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# Journal of Nepal Agricultural Research Council

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**ACKNOWLEDGMENTS:** Acknowledge the person/s and/or institution/s or funding agencies, if necessary, who actually help to achieve the objectives of the research.

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tillers allowing penetration of sunlight and good aeration. The flag leaves are straight up-right with length of more than 20 cm above panicles. These morphological characters allow less contact among tillers, thus negate disease spread. This is probably the reason that *Sabitri* and *Tetep* were extensively used as donor parents in breeding program considering plant morphology as a model in selecting the genotypes with similar plant stature for improving ShB resistance in rice.

**Table 3.** Resistance in rice genotypes to sheath blight under field conditions in 2011 wet season

| IRRI accession | Genotype | Disease incidence (%) | | | AUDPC‡ |
|---|---|---|---|---|---|
| | | 14 DAI | 21 DAI | 28 DAI† | |
| IRGC-32576 | TETEP | 0 | 0 | 13.33 G | 46.66 H |
| IEGC-24154 | IR 26 | 0 | 0 | 16.13 FG | 56.45 GH |
| IR86174-17-15-11-23 | SHB-134-11-23 | 0 | 1.852 | 19.16 FG | 80.04 F-H |
| MTU-1010 | MTU-1010 | 1.852 | 1.515 | 20.69 FG | 89.50 E-H |
| IRGC-39723 | RNR 57979 | 0.952 | 5.119 | 16.07 FG | 95.40 D-H |
| IR86174-17-5-18-45 | SHB-129-18-45 | 1.667 | 4.227 | 17.72 FG | 97.46 D-H |
| IRGC5565 | SABITRI | 1.389 | 4.568 | 17.56 FG | 98.31 D-H |
| IRGC-39932 | SI 51 | 2.279 | 3.262 | 21.98 E-G | 107.8 C-H |
| IRGC-19907 | IR 24 | 0 | 5.681 | 20.39 FG | 111.1 C-H |
| IR86174-17-15-11-44 | HSB-134-11-44 | 0 | 6.277 | 20.07 FG | 114.2 C-H |
| IR86174-17-15-11-42 | SHB-134-11-42 | 3.03 | 8.73 | 12.73 G | 116.3 C-H |
| IRGC-77301 | | 2.646 | 4.884 | 22.66 E-G | 122.8 C-H |
| RTP11793 | RNP74229 | 0.813 | 6.389 | 22.29 E-G | 125.6 C-H |
| IRGC-38624 | BR 3 | 2.464 | 7.898 | 21.48 E-G | 139.1 C-H |
| IRGC-40020 | LANKANDA 41 | 3.171 | 6.624 | 24.89 C-G | 144.6 B-H |
| IRGC-47111 | RAJESHWARI | 0 | 5.808 | 33.09 B-G | 156.5 B-H |
| IRGC-117278 | SWARNA | 1.235 | 10.345 | 23.72 D-G | 159.7 B-H |
| IRGC-59244 | TARA | 1.235 | 11.765 | 28.37 B-G | 186.0 B-H |
| IRGC-53200 | MATAHAMBRE | 3.889 | 13.041 | 40.52 B-G | 246.7 B-H |
| IRGC-6264 | N-22 | 1.389 | 16.186 | 41.86 B-G | 264.7 B-G |
| IRGC77471 | YUEFU | 5.635 | 15.588 | 40.92 B-G | 272.1 B-F |
| IRGC116438 | AZ(C) | 0 | 16.667 | 44.74 B-G | 273.3 B-F |
| IRGC-117398 | VANDANA | 2.564 | 9.804 | 63.09 A-D | 298.4 B-E |
| IRGC-4819 | N-22 | 4.312 | 9.283 | 63.72 A-C | 303.1 B-D |
| IRGC-74540 | TOMOE MOCH | 3.935 | 11.997 | 61.11 A-E | 311.6 BC |
| IRGC-19379 | N 22 | 0 | 16.987 | 56.02 A-F | 315.0 BC |
| IRGC-62172 | KAMMRA | 0 | 4.762 | 90.20 A | 349.0 AB |
| IRGC82775 | IAC 165 | 6.278 | 36.84 | 67.42 AB | 515.8 A |
| $LSD_{0.05}$ | | NS | NS | 33.47 | 172.3 |

† Values followed by different letters are significantly different at (P = 0.05)
‡ Values followed by different letters within the column are significantly different at (P = 0.05)

## ACKNOWLEDGEMENTS

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# Genetic Parameters of Common Wheat in Nepal

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## ABSTRACT

Knowledge on variation within traits and their genetics are prerequisites in crop improvement program. Thus, in present paper we aimed to estimate genetic and environmental indices of common wheat genotypes. For the purpose, eight quantitative traits were measured from 30 wheat genotypes, which were in randomized complete block design with 3 replicates. Components of variance and covariance were estimated along with heritability, genetic gain, realized heritability, coheritability and correlated response. Differences between phenotypic and genotypic variances in heading days, maturity days and plant height were not large. Grain yield and plant height showed the highest phenotypic (18.189%) and genotypic (12.06%) coefficient of variances, respectively. Phenotypic covariance was higher than genotypic and environmental covariance in most of the traits. The highest heritability and realized heritability were of heading days followed by maturity days. Genetic gain for plant height was the highest. Co-heritability of 1000-grain weight with tillers number was the highest. The highest correlated response was expressed by grain yield with tillers number. This study indicates the possibility of improving wheat genotypes through selection utilizing existing variation in these traits.

**Key words:** Covariance components, genetic parameters, *Triticum aestivum*, variance components

## सारांश

बालीको जातीय विकासको लागि वंशाणुगत गुणहरुको विविधता र यी गुणहरुको अनुवंश बारे अध्ययन गर्नु आवश्यक हुन्छ । वंशाणुगत गुणहरु बारे विभिन्न आठ वटा गुणहरुको ३० वटा गहुँको जातहरुमा आर.सी.वि.डी. प्रयोग गरि तीन छुट्टाछुट्टै सेटमा वंशाणुगत गुणहरुको मात्रा जस्तै genetic gain, heritability, coheritability, variance-covariance components आदिको अध्ययन गरिएको थियो । फूल फुल्ने अवधि, पाक्ने अवधि र बोटको उचाईमा phenotypic /genotypic विविधता बीच धेरै फरक नभएको पाइयो । सबै भन्दा बढी phenotypic coefficient of variance (१८.१८%) गहुँको दानाको तौलमा र genotypic coefficient of variance (१२.०६%) बिरुवाको उचाईमा पाइयो । अधिकतर गुणहरुमा genotypic / environmental covariances भन्दा phenotypic covariance बढी भएको देखियो । फुल फुल्ने र पाक्ने अवधि जस्ता गुणहरुको heritability र realized heritability सबै भन्दा बढी पाइयो । सबै भन्दा बढी आनुवंशिक उपलब्धि बालीको उचाईमा थियो । हजार दानाको तौल, tiller संख्या संगको coheritability सबै भन्दा बढी थियो । बढी correlated response दानाको तौल र tiller संख्याले देखायो । यस अध्ययन बाट उक्त जातहरुमा आनुवंशिक विविधता भएको पाइएकोले उक्त जातहरु बाट नयाँ जात निकाल्न सकिने सम्भावना देखिन्छ ।

## INTRODUCTION

Varietal richness both in terms of landraces and modern variety including introduced and wild genotypes of wheat is high in Nepal (NARC 1997, Mudwari 1999, Upadhaya and Joshi 2003, Joshi et al 2004, Mudwari et al 2004, Joshi et al 2013). Altitudinal climatic variation and farmers' needs are probably the major attributers for high wheat diversity. Diversity study among quantitative traits and their genetic parameters estimates are prerequisite in wheat breeding program (Desheva and Kyosev 2015, Farshadfar and Estehghari 2014, Farshadfar et al 2013). Use of genetic resources requires their proper and systematic evaluation due to substantial variation of wheat varieties in India (Pathak and Nema 1985), Ethiopia (Belay et al 1993), Nepal (Joshi et al 2004), Iran (Farshadfar and Estehghari 2014), Bulgaria (Desheva and Kyosev 2015) and many other countries. Murty and Arunachalam (1966) stated that genetic drift and selection in different environments could cause greater diversity than geographical distance. Natural selection super-imposed by human under varied agro-climatic, soil and stress environmental conditions have produced marked variability that can be utilized for new varieties improvement and development (Murty and Arunachalam 1966). Currently, such characterization has been more crucial in the context of climate resilient varieties and resistant not only to diseases but also to drought (Desheva and Kyosev 2015, Farshadfar and Estehghari 2014). It should be recognized that improvement in one character as a result of selection for another depends not only on the genotypic and phenotypic correlations, but also on the genotypic and phenotypic variances associated with them (Johnson et al 1955).

Wheat is the third most important crop after rice and maize in Nepal. Different approaches have been employed to increase the productivity of wheat. Genetic improvement is the major technique to develop high yielding including drought tolerance and climate resilient varieties (Farshadfar et al 2013). Estimates of genetic parameters are important to efficiently breed the wheat. Information on genetic parameters is useful to plant breeders in order to design efficient breeding methods for crop improvement program. Considering these gaps, the objective of present study was to measure variance and covariance components, phenotypic and genotypic coefficients of variations, heritability ($h^2$), coheritability ($ch^2$), realized heritability ($rh^2$) correlated response (CRy) and genetic gain ($\Delta G$), for selected important quantitative traits of wheat.

## MATERIALS AND METHODS

Altogether 30 wheat genotypes (28 advanced lines and two-released cultivars) were evaluated in Initial Evaluation Trial at Agriculture Botany Division (ABD) in Khumaltar, Kathmandu valley in 2008. The ABD is located at 1350 m above the sea level. The climate of the experimental site is warm temperate mid hill agro-ecological condition. Some of these wheat genotypes were received from

International Maize and Wheat Improvement Center (CIMMYT), Mexico and some were developed by Agriculture Botany Division. Standard agronomical practices were followed using randomized complete block design with three replications. Eight traits namely heading days, maturity days, plant height, tillers number, grains number per spike, grains weight per spike, 1000-grain weight and grain yield were measured based on the descriptors (IBPGR 1985).

Based on these replicated data, variance, covariance components, and coefficients were estimated. Genetic parameters (heritability, genetic advance, genetic gain, selection differential, realized heritability, coheritability and correlated response) were computed. These were estimated following the procedures of Mudwari (1985), Johnson et al (1955), Robinson et al (1949), Burten and De Vane (1953) and Singh and Chaudhary (1985). Data were processed in MS Excel and analyzed in MINITAB and AGROBASE software.

## RESULTS

The highest genetic coefficient of variation (GCV) was recorded for plant height (12.06%) followed by grain yield (9.92%), while lowest GCV was observed for headings and maturity days (Table 1). Phenotypic coefficient of variance (PCV) was usually higher than GCV, but the difference was very low (Table 1). The higher GCV and PCV for grain yield were (9.926% and 18.169%), plant height (12.06% and 12.96%) and tillers number (9.67% and 14.89%), respectively. The highest PCV was shown by grain yield (18.169%) followed by grain weight per spike (17.199%) (Table 1).

The heritability ($h^2$) estimate varied from 16% for grain weight/spike to 94% for heading days (Table 2). Moderate to high heritability estimates were found for 1000-grain weight, tillers number, and plant height. The estimate of $h^2$ for grain yield, grain weight/spike and grain number were per spike less. The genetic gain expressed as a percentage of the mean, ranged from 5.185 for maturity days to 23.104 for plant height. Heading days, maturity days, grain number/spike and grain weight per spike had relatively low values (Table 2).

To assess the tendency of various quantitative traits values for genotypic, phenotypic and environmental components, covariance and coheritability of 30 genotypes were determined (Table 3 and 4). Though most of the traits were inherited together, tiller numbers exhibited much higher values of coheritability with grain number per spike and 1000-grain weight. High coheritability was estimated amongst the traits especially grain yield reflected closer association and lesser environmental influence. The changes brought about through indirect selection ie correlated response (CRy) on an associated trait are given in Table 5. The highest CRy value was shown by grain yield with tillers number.

**Table 1.** Variance components of phenotype, genotype and environment and coefficient of variation with mean of 30 wheat genotypes (Symbols: Vp, Phenotypic variance. Vg, Genotypic variance. Ve, Environmental variance. PCV, Phenotypic coefficient of variation. GCV, Genotypic coefficient of variation)

| Trait | Vp | Vg | Ve | PCV, % | GCV, % | Mean |
|---|---|---|---|---|---|---|
| Heading days | 23.187 | 21.821 | 1.366 | 4.118 | 3.994 | 116.944 |
| Maturity days | 18.017 | 16.355 | 1.662 | 2.773 | 2.642 | 153.078 |
| Plant height, cm | 142.820 | 123.520 | 19.300 | 12.968 | 12.060 | 92.156 |
| Tillers number | 3017.333 | 1274.333 | 1743.000 | 14.891 | 9.677 | 368.878 |
| Grain number/spike | 32.087 | 5.297 | 26.790 | 15.754 | 6.401 | 35.956 |
| Grain wt/spike, g | 0.080 | 0.013 | 0.068 | 17.199 | 6.807 | 1.648 |
| 1000-grain wt, g | 21.383 | 10.983 | 10.400 | 10.073 | 7.220 | 45.905 |
| Grain yield, kg/ha | 207297.333 | 61866.333 | 145431 | 18.169 | 9.926 | 2505.871 |

**Table 2.** Genetic parameters estimated from 30 wheat genptypes

| Trait | Heritability ($h^2$) | Realized heritability ($rh^2$) | Expected genetic advance (GA) | Actual Genetic gain ($\Delta G$) | Selection differential (S) |
|---|---|---|---|---|---|
| Heading days | 0.941 | 0.941 | 9.335 | 7.983 | 8.482 |
| Maturity days | 0.908 | 0.907 | 7.937 | 5.185 | 5.712 |
| Plant height | 0.865 | 0.864 | 21.292 | 23.104 | 26.714 |
| Tillers number | 0.422 | 0.422 | 47.790 | 12.956 | 30.675 |
| Grain number/spike | 0.165 | 0.165 | 1.926 | 5.357 | 32.453 |
| Grain wt/spike | 0.157 | 0.156 | 0.091 | 5.549 | 35.430 |
| 1000-grain wt | 0.514 | 0.513 | 4.893 | 10.659 | 20.751 |
| Grain yield | 0.298 | 0.298 | 279.914 | 11.170 | 37.428 |

**Table 3.** Phenotypic, genotypic and environmental covariance estimated from 30 wheat genotypes

| Trait | | 2 | 3 | 4 | 5 | 6 | 7 | Grain yield |
|---|---|---|---|---|---|---|---|---|
| Heading days | $P_{cov}$ | 16.060 | -13.730 | -26.325 | 0.907 | 0.165 | 0.768 | -108.128 |
| | $G_{cov}$ | 15.344 | -13.559 | -16.331 | 0.233 | 0.014 | 0.489 | 2.754 |
| | $E_{cov}$ | 0.716 | -0.171 | -9.995 | 0.674 | 0.151 | 0.279 | -110.882 |
| Maturity days | $P_{cov}$ | | -18.891 | -47.643 | 5.187 | 0.107 | -0.445 | 144.139 |
| | $G_{cov}$ | | -20.395 | -37.831 | 5.458 | 0.124 | 0.313 | 191.899 |
| | $E_{cov}$ | | 1.504 | -9.812 | -0.270 | -0.017 | -0.759 | -47.760 |
| Plant height | $P_{cov}$ | | | -71.626 | -5.595 | 0.564 | 9.378 | -280.233 |
| | $G_{cov}$ | | | -112.209 | -8.617 | 0.383 | 8.745 | -775.469 |
| | $E_{cov}$ | | | 40.583 | 3.021 | 0.180 | 0.633 | 495.236 |
| Tillers number | $P_{cov}$ | | | | -21.446 | 2.144 | -4.199 | 13753.840 |
| | $G_{cov}$ | | | | -61.458 | -2.100 | -27.371 | 5871.005 |
| | $E_{cov}$ | | | | 40.012 | 4.244 | 23.172 | 7882.835 |
| Grain number/spike | $P_{cov}$ | | | | | 1.479 | -4.625 | 273.031 |
| | $G_{cov}$ | | | | | -0.063 | -3.368 | 33.298 |
| | $E_{cov}$ | | | | | 1.542 | -1.257 | 239.733 |
| Grain wt/Spike | $P_{cov}$ | | | | | | 0.485 | 47.818 |
| | $G_{cov}$ | | | | | | 0.258 | 14.646 |
| | $E_{cov}$ | | | | | | 0.227 | 33.172 |
| 1000-grain wt | $P_{cov}$ | | | | | | | 189.764 |
| | $G_{cov}$ | | | | | | | 312.688 |
| | $E_{cov}$ | | | | | | | -122.924 |

$P_{cov}$, Phenotypic covariance. $G_{cov}$, Genotypic covariance. $E_{cov}$, Environmental covariance

**Table 4.** Coheritability ($ch^2$) among quantitative traits in 30 wheat genotypes

| Trait | Heading | Maturity | Plant | Tillers | Grain | Grain | 1000-grain | Grain yield |
|---|---|---|---|---|---|---|---|---|
| Heading days | | 0.955 | 0.988 | 0.620 | 0.257 | 0.087 | 0.637 | -0.025 |
| Maturity days | | | 1.080 | 0.794 | 1.052 | 1.159 | -0.703 | 1.331 |
| Plant height | | | | 1.567 | 1.540 | 0.680 | 0.932 | 2.767 |
| Tillers number | | | | | 2.866 | -0.979 | 6.519 | 0.427 |
| Grain | | | | | | -0.043 | 0.728 | 0.122 |
| Grain wt/spike | | | | | | | 0.532 | 0.306 |
| 1000-grain wt | | | | | | | | 1.648 |

**Table 5.** Correlated response (CRy) among quantitative traits in wheat

| Trait | Heading days | Maturity days | Plant height | Tillers number | Grain number/spike | Grain wt/spike | 1000-grain wt | Grain yield |
|---|---|---|---|---|---|---|---|---|
| Heading days | | 6.564 | -5.800 | -6.986 | 0.099 | 0.006 | 0.209 | 1.178 |
| Maturity days | | | -9.898 | -18.360 | 2.649 | 0.060 | 0.152 | 93.131 |
| Plant height | | | | -6.022 | -0.462 | 0.021 | 0.469 | -41.616 |
| Tillers number | | | | | -2.305 | -0.079 | -1.026 | 220.175 |
| Grain number/spike | | | | | | -0.023 | -1.225 | 12.109 |
| Grain wt/spike | | | | | | | 1.875 | 106.456 |
| 1000-grain wt | | | | | | | | 139.297 |

## DISCUSSION

The magnitude of heritable variability, more particularly its genetic component is the most important aspect of the genetic constitution of the breeding material, which has a close bearing on the response to selection. For this determination, it was necessary to divide the total phenotypic variance of the quantitative traits into its components as these are the basis for genetic analysis, because the dimensions of these components decide the breeding behavior of the population and provide the bases of selection techniques (Moghaddam et al 1998). The contribution of environmental factors was substantially important on total variance because of the lower values of GCV in comparison to PCV. Farshadfar and Estehghari (2014), Desheva and Kyosev (2015) also reported similar result. GCV gives a quantitative measurement of genetic variability in a particular trait. The highest genetic coefficient of variation in plant height followed by grain yield indicates the effective selection for these traits. Heading days (3.99%) and maturity days (2.64%) exhibited lower values of GCV suggesting that these traits are influenced by environment. Therefore, the selection for these traits will not be effective. Similar results were also obtained by Mudwari (1985). Since, most of these traits are controlled polygenically and inherited quantitatively, thus substantial environmental influence might impact on these traits. Maturity days, heading days, plant height and tillers number were exhibited higher genetic variance than environmental variance (Table 1) indicating thereby a lesser influence of environment upon these traits. These traits had also high heritability values (Table 2).

High genotypic variance for a particular trait may not reflect heritable proportion of variation. Estimates of variance and its components alone are not helpful in determining the heritable portions of variation (Falconer 1960). For this, an estimation of heritability ($h^2$), the heritable proportion of variances of the traits seems necessary. High genetic gain was observed for plant height, tillers number, 1000-grain weight and grain yield. Similar results were observed by Moghaddam et al (1998) confirming the validity estimates of present results.

In the present study, the estimate of $h^2$ was large for 1000-grain weight (0.514), plant height (0.865), heading days (0.941), low for grain yield (0.298) and grain number (0.165) (Table 2). These values were similar to the findings of Sikka and Jain (1958) showing the validity of the findings. Bhullar et al (1982) in genetic analysis of durum wheat reported low $h^2$ estimates for grain yield, spikes per plant, medium for spikelets per spike, grain per spike, 1000-grain weight and plant height. Heritability estimates reported for grain yield in wheat were usually low (Gandhi et al 1964, Pathak and Nema 1985, Mudwari 1985). High GCV value with intermediate estimates of $h^2$ and genetic gain in this study agreed with Belay et al (1993).

Since environmental variance is dependent upon the conditions of culture or management, more variable (fluctuating) conditions reduce $h^2$, contrarily higher uniformity increases it. The values of $h^2$ computed in this study for wheat refer to the populations grown in Khumaltar, Kathmandu Valley or similar condition elsewhere to great extent. However, such values for other populations in other situations could be liable to be more or less similar according to the population and the environmental conditions. The genotype-environment interaction variance was not separated from the genetic variance in this study, therefore the estimates of GCV, $h^2$ and genetic gain might be inflated.

Genetic gain along with high $h^2$ is more useful in predicting the resultant effects of selection (Johnson et al 1955). Plant height exhibited the highest genetic gain, whereas tillers number, 1000-grain weight and grain yield showed substantially high response to selection (Table 2). Three characters i.e. plant height, tillers number and 1000-grain weight exhibiting high $h^2$ along with higher genetic gain suggests that these characters can also be improved by selection. The low genetic gain against high $h^2$ estimate for heading days and maturity days showed that the expression of these characters were dependent to non-heritable variability up to certain extent.

The expected genetic advance (GA) was the highest on grain yield, but the highest genetic gain was observed on plant height (Table 2). The gain on grain yield from selection expressed as a percentage of the mean was 11.17%. This means that if the top 5% of the wheat plants were chosen, the grain yield would be expected to increase by 11.17% after one cycle of selection.

Grain yield, 1000-grain weight, tillers number and plant height possessed substantially higher genetic variability and also exhibited higher genetic gains revealing greater response to the selection. These observations agreed with the results reported by Belay et al (1993) and Moghaddam et al (1998). A low GCV and low GA were observed for heading days and maturity days (Table 1 and 2). These indicated that the characters were under high environmental influence thus, selection based on these characters would be ineffective. The high value of GCV, $h^2$ and GA estimated for plant height, tillers number and 1000-grain weight indicated the predominance of additive gene action suggesting direct phenotypic selection based on these traits would be effective for varietal improvement.

The $h^2$ and $rh^2$ values were similar in four traits and $rh^2$ was slightly lower than $h^2$ in others four traits (Table 2). The $rh^2$ is primarily a description of the response and may not provide a valid estimate of the $h^2$ in the base population (Falconer, 1960). Larger population tends to give response to selection due to mainly to larger realized $h^2$. Days to maturity and 1000-grain weight have played important role to increase grain yield because of high genetic covariance of these traits with grain yield (Table 3). Selection based on the number of tillers and grain number per spike could not be considered effective to increase yield due to high environmental covariance. High genotypic covariance between grain yield and weight was also reported by Farshadfar and Estehghari (2014). High values of genotypic covariance are very useful in breeding program to improve the traits simultaneously. Coheritability estimates were positive for most of traits (Table 4). The high coheritability values of grain yield with maturity days, plant height and 1000-grain weight indicate that selection on these traits would affect largely on grain yield. Farshadfar and Estehghari (2014) observed high coheritability values of grain yield with other agro-morphological traits.

If the trait under selection is genetically correlated with other trait(s), one can expect improvement in the correlated trait(s) as well. Thus, improvement in a trait can be made by direct as well as indirect selection. If we consider a plant height as selection criteria, negative indirect result will be observed in grain yield (Table 5). Because of the linkage relationship, correlated response frequently occurs under selection.

Knowledge of the genetic parameters of a population is useful in designing an effective breeding program. High $h^2$ and GCV (and therefore high genetic advance) are positive indication that the variation in these traits is attributable to a high degree of additive genetic effect. Selection for these characters should be effective and satisfactory for practical purpose. Information on these aspects will be valued by wheat breeders.

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# Soil Physico-Bio-Chemical Properties under Poplar + Indian Mustard Inter Cropping System

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## ABSTRACT

A field experiment was conducted during the winter seasons of 2008-10 at Agroforestry Research Centre, Pantnagar, India with aim to examine the effect of different levels of recommended Nitrogen (N): Phosphorus (P): Potassium (K) (NPK) on soil physico-bio-chemical properties under varying poplar tree densities with mustard introcropping. Lower soil bulk density was recorded under 1000 trees/ha density compared to sole crop in both the year. Soil bulk density (BD) decreased underneath trees. However, soil pH, available N and K were not influenced by tree density in both the years. Electrical conductivity (EC) and soil organic carbon (SOC) increased underneath trees of either tree density compared to sole crop in both the years. Significantly ($P<0.05$) higher available P was recorded under 1000 trees/ha density compared to 250 and 500 trees/ha densities including sole crop in 2008-09. Similarly, soil microbial biomass carbon (SMBC) increased with increasing the tree density and significantly ($P<0.01$) higher value was recorded under 1000 trees/ha density compared to sole crop and sparse density both the years except 2009-10, where 500 and 1000 trees/ha densities remain statistically at par. On the other hand, dehydrogenase activity (DA) was maximum under 500 trees/ha density compared to sole crop and 1000 trees/ha density in both the years. Among the fertility levels, the maximum SOC and available N were recorded with 75% compared to 50, 100 and 125% of recommended NPK in 2008-09, except available N with 100% of recommended NPK. But, available P was more with 100% of recommended NPK in 2008-09. Similarly, maximum SMBC were recorded with 75 % compared to higher doses of recommended NPK in both the years. Similarly, BD, EC, SOC, available N, P and K were recorded maximum and soil pH minimum in 0-15 cm soil layers compared deeper layers.

**Key words**: Fertility levels, pH, available N, P and K, DA

## सारांश

माटोको भौतिक, जैविक तथा रसायनिक गुणमा विभिन्न सिफारिश मात्राको नाइट्रोजन, फास्फोरस तथा पोटासको लहरे पीपलका विभिन्न घनत्व र खुला स्थानमा परीक्षण गर्ने उद्देश्यले सन् २००८ देखी २०१० को हिउँदे मिजनमा तेलवाली लगाई गोविन्दवल्लभ पंत कृषि तथा प्रोद्योगिकी विश्वविद्यालय, भारतको कृषि वन अनुसन्धान केन्द्र, पथ्थर चट्टामा परीक्षण संचालन गरिएको थियो । माटोको खदिलोपन (स्वायल वल्क डेन्सिटी) लहरे पिपलका रुखहरु मुनि घटेको र १००० रुख प्रति हेक्टर लगाइएको खेतमा रुख नभएको खाली खेत (एकलवाली) को तुलनामा तात्विक रुपमा दुवै वर्ष कम मापन गरिएको थियो । यद्यपि माटोको अम्लियपन, उपलब्ध नाइट्रोजन तथा पोटासियमको मात्रा दुवै वर्षमा विभिन्न घनत्वका रुखले प्रभावित पारेको पाईएन । विधुतिय चालकता (इलेट्रीकल कन्डकटिभिटी) तथा माटोको जैविक कार्वन दुवैवर्ष सवै घनत्वका रुखहरु मुनि खाली खेतको तुलनामा बढेको पाइयो । सन् २००८ र ०९ मा माटोको उपलब्ध फास्फोरसको मात्रा १००० रुख प्रति हेक्टरको घनत्वमा २५० र ५०० रुख प्रति हेक्टर र खाली खेत भन्दा तात्विकरुपले बढी मापन गरियो । त्यसैगरी, माटोको सुक्ष्म जिवाणुहरुको जैविककार्वन (स्वायल माइक्रोवियल वायोमास कार्वन) लहरे पिपल रुखका घनत्वहरु बढदै जाँदा बढेको पाइयो र खाली खेत र पातलो घनत्व भएका रुखहरुको खेतमा भन्दा १००० रुख प्रति हेक्टर भएको खेतमा बढी मापन गरियो । अर्को तर्फ दुवै वर्ष डिहाड्रोजेनेज एक्टीभिटी (इनजाइमेटिक गतिविधि) ५०० रुख प्रति हेक्टर घनत्व भएको खेतमा, खाली खेत र १००० रुख प्रति हेक्टर भएको खेतमा भन्दा बढी रहेको पाइयो । २००८ र ०९ मा सवभन्दा बढी माटोको प्राङ्गारिक कार्वन र उपलब्ध नाइट्रोजनको मात्रा ५०, १०० र १२५५ भन्दा ७५५ सिफारिश मात्राको नाइट्रोजन, फास्फोरस, पोटासियम प्रयोग गरेको खेतमा पाइयो । तर, २००८ र ०९ मा उपलब्ध फास्फोरस १००५ सिफारिस मात्राको नाइट्रोजन, फास्फोरस, पोटासियम प्रति हेक्टर दिएको खेतमा बढी मापन गरियो । त्यसै गरी, माटोको सूक्ष्म जैविककार्वन पदार्थ उच्च मात्रामा भन्दा ७५५ सिफारिश मात्राको नाइट्रोजन, फास्फोरस, पोटासियम प्रति हेक्टर दिएको खेतमा दुवै वर्ष बढी मापन गरियो । माटोको घनत्व, विधुतिय चालकता, माटोको जैविक कार्वन, उपलब्ध नाइट्रोजन, फास्फोरस, पोटासियम जस्ता परिवर्त्यहरु (Variables) माथिल्लो सतहको माटो (०-१५ से.मी.) मा तल्लो सतहको माटोमा भन्दा बढी मापन गरियो भने माटोको अम्लियपना माथिल्लो सतहमा कम रहेको पाइयो ।

## INTRODUCTION

Soil fertility deterioration, in general, and organic matter depletion in particular under intensive agriculture system are the main problems in the tropical and sub-tropical region of Indian sub-continent. To certain extent agro-forestry land use system could be an alternate to reduce land degradation. Recently, Chauhan et al (2010) suggested Poplar *(Populus deltoides* Bartr) based agro-forestry as one of the viable land use options to prevent land degradation by which biological production could be restored sustainably. This system has been quite popular in northern India with expanding coverage every year. In such a system organic carbon content in soil (0-15 cm) has been shown to increased by 35.6 % after 6 years of poplar planting than in land having single wheat crop (Chauhan et al 2010). As such, substantial loss in wheat yield occurred under Poplar, but compensated by the trees in terms of biomass, economics and carbon mitigation potential. There is need to increase the level of soil organic carbon not only to maintain land productivity, but also to provide a sink for atmosphere $CO_2$ in the terrestrial carbon pool. Moreover, the deposition of Poplar leaf litter changes C:N (Carbon:Nitrogen) ratio of the soil compensating potential crop growth.

The leaf litter biomass may vary depending on the tree age, density and management, hence nutrient cycling may also vary because soil biological activities are influenced by trees canopy, thereby availability of nutrients to underneath crops (Pingale 2009). The graded levels of fertilizers applied in inter cropping system invariably used by both crop as well as trees in agri-silvicultural system (Prasadini and Sreemannarayana 2007). However, such knowledge has rarely been examined in specific agri-silviculture system. Therefore, the present paper attempt to provide empirical knowledge on residual effect in soil in terms of physical, chemical and biological properties having varying Poplar tree density underneath Indian mustard with various levels of fertilizers applied.

## MATERIALS AND METHODS

The field experiments were conducted at Agroforestry Research Centre Patharchatta of Govind Bhallav Pant University of Agriculture and Technology, Pantnagar, Uttarakhand located at 29$^0$N Latitude, 79.3$^0$E Longitude and at an altitude of 243.84 m above mean sea level during 2008-09 and 2009-10. The Poplar clone G4 were planted during February 2000 and mortality was maintained by gap-filling in following year to maintain 16 trees in each density at five meter row distance. The soil of the experiment site was clay loam (0-15 cm) in texture, having pH 8.0-8.05, organic carbon 0.99-1.17%, available nitrogen (N) 143.3-141.4 kg/ha, available phosphorus (P) 11.7-12.7 kg/ha and available potassium (K) 133.9-136.7 kg/ha in 0 to 45 cm soil layer under treeless (sole crop) to 1000/ha tree density plots. The land was thoroughly ploughed and incorporated leaf litter into the soil two weeks before sowing. Upland rice and wheat cropping sequence were followed till the beginning of the present experiment. The field remained fallow between the two experiments in *kharif* seasons of 2008-09 and 2009/10. The experiment was laid out in split-plot design taking four tree densities, i.e. Sole crop (open), 250, 500 and 1000 trees/ha plots with four fertility viz. 50%, 75%, 100% and 125% of recommended dose of fertilizer (RDF) for Indian mustard (*Brassica juncea* L.) as sub-plot treatment with four replications. The recommended fertilizer dose was 120 kg N, 40 kg $P_2O_5$ and 20 kg $K_2O$/ha. The NPK mixture (12:32:16) and urea (46% N) were used as a source of fertilizers in both the years. The doses of 50% N and 100% P and K for Indian mustard were applied into the soil at the time of sowing.

Indian mustard var. Kranti was inter-cropped on 8$^{th}$ Nov in 2008 and 7$^{th}$ Nov in 2009. The mustard seed was sown in lines 30 cm apart continuously keeping seed rate @ 5 kg/ha manually. The crop was irrigated only once with tube well water in both years at about 45 days after sowing (DAS). The remaining 50% N was top dressed after one week of irrigation. The crop was harvested on March 23$^{rd}$ and 26$^{th}$, in 2009 and 2010, respectively when more than 80% pod turned yellow. Soil samples from each main plot in 0-15, 15-30 and 30-45 cm depths were collected before sowing and after harvest of mustard crop in both the years for assessing chemical properties viz. pH, Electrical Conductivity (EC), Soil Organic Carbon and available N, P and K.

-For soil microbial study, samples from each subplot at 0-15 cm soil depth were collected at peak flowering stage and stored in deep freeze for the determination of soil microbial biomass carbon and dehydrogenase activity. Bulk density was determined by Core sampler from each main plot at 0-15, 15-30 and 30-45 cm soil depths before sowing mustard crop. Soil samples were analyzed for pH (Jackson 1973), EC, SOC (Black 1965), available N (Subbiah and Asija 1956), P (Olsen et al 1954) and K (Jackson 1973) using standard procedures. The soil values of pH, EC, SOC, available N, P and K were determined depth wise in 0-15, 15-30 and 30-45 cm depths. Chloroform fumigation extraction (Jenkinson and Powlson 1976) and TTC assay method (Tabatabai 1994) were employed for the determination of soil microbial biomass carbon (SMBC) and dehydrogenase activity (DA), respectively.

## RESULTS

### Soil Physical Properties

#### *Soil Bulk Density*

The soil bulk density (BD) (g/cc) was influenced by tree density and soil depths in both the years, respectively. The soil bulk density decreased with increasing tree density (Table 1). The highest 1.498 g/cc soil bulk density was recorded in sole cropping (open plot) as compared to underneath trees density in 2008-09 and 2009-10, respectively. However, the differences in soil bulk density between 250 and 500, and 500 and 1000 trees/ha plots in first year; and amongst 250, 500 and 1000 trees/ha densities in the second year were insignificant. The soil bulk density increased with increase in the soil depth. However, 15-30 cm soil depth had significantly ($P<0.05$) higher bulk density than 0-15 cm in 2009-10 (Table 1).

### Soil Chemical Properties

#### *Soil pH*

The soil pH after harvest of mustard crop was not influenced by tree density and fertility levels both during 2008-09 and 2009-10, while the soil pH varied with soil depths in both the years (Table 2). The soil pH increased with increasing the soil depth up to 30 cm and declined in further deeper (30-45 cm). The higher (8.04 and 8.47) and lower (7.80 and 8.23) soil pH was observed in 15-30 and 0-15 cm soil depths, respectively in both the years.

#### *EC of Soil*

The EC (dS/m) of extract from saturated soil after harvest of mustard crop was influenced by tree density, fertility levels and soil depths in both the years (Table 2). The EC of soil increased with increased tree density. The maximum (0.610 and 0.508 dS/m) substantially higher EC was recorded under 500 trees/ha density in 2008-09 and under 1000 trees/ha density in 2009-10, respectively, as compared to sole crop (open plot) and 250 trees/ha density in 2008-09. EC was similar in open plot and sparse density (250 trees/ha) in first year and higher density in second year. Moreover, EC of soil underneath poplar trees was more compare to sole crop (open plot) in both the years. Among the fertility levels, the maximum (0.678 and 0.480 dS/m) EC was recorded with 50% compared to 75, 100 and 125% of recommended NPK in first year and with 100% compared to 75 and 125% of recommended NPK in second year, respectively. Whereas, the differences between 50 and 100% and 75 and 125% of recommended NPK in 2009-10 were not significant. The EC of soil decreased with increasing soil depth and the maximum (0.588dS/m) EC was recorded in 0-15 cm depth comparing to 15-30 and 30-45 cm layers in 2008-09 and 2009-10. However, the EC in 15-30 and 30-45 cm soil depths were almost similar in 2009-10.

**Table 1.** Soil bulk density as influenced by poplar tree density and varying soil depths

| Treatment | Bulk density (g/cc) | |
|---|---|---|
| **A. Tree density (trees/ha)** | **2008-09** | **2009-10** |
| Sole crop | 1.402 | 1.498 |
| 250 | 1.328 | 1.389 |
| 500 | 1.313 | 1.389 |
| 1000 | 1.295 | 1.375 |
| SEm ± | 0.009 | 0.024 |
| CD, 5% | 0.030 | 0.078 |
| **B. Soil depth (cm)** | | |
| 0-15 | 1.290 | 1.293 |
| 15-30 | 1.366 | 1.414 |
| 30-45 | 1.349 | 1.531 |
| SEm ± | 0.008 | 0.023 |
| CD, 5% | 0.024 | 0.069 |

### *Organic Carbon (OC) Content in Soil*

The organic carbon (%) of soil after harvest of mustard crop was influenced by tree density, fertility levels and soil depths in both the years, except by fertility levels in 2009-10 (Table 2). The SOC (%) increased with increasing tree densities having maximum values 1.11 and 1.01% in 2008-09 and 2009-10, respectively. The highest OC was recorded under highest density (1000 trees/ha) in both years, compared to sole crop field. The differences between sole crop and 250 trees/ha and amongst 250, 500 and 1000 trees/ha densities in first year and 250, 500 and 1000 trees/ha densities in second year were somewhat closer. The SOC increased by 11 and 25%; 15 and 26% and 19 and 26% with each successive increase in tree densities from zero to 1000/ha in 2008-09 and 2009-10, respectively.

Among the fertility levels, the maximum (1.11%) soil OC was recorded with 75% of recommended NPK compared to 50, 100 and 125 % in 2008-09. The differences between 50, 100 and 125% of recommended NPK were not substantial with respect to SOC content in soil. Organic carbon of soil decreased with increasing soil depth having maximum (1.41%) recorded in 0-15 cm depth comparing to 15-30 and 30-45 cm depths in both years. However, 15-30 cm depth had higher SOC than 30-45 cm in both the years. Surface soil layer (0-15 cm) showed approximately two times (107 and 133%) more SOC. Whereas, the middle soil layer (15-30 cm) contained 53 and 45% more SOC than the lower most studied layer i.e. 30-45 cm.

### *Available Nitrogen in Soil*

The available N in soil after harvest of mustard crop was not influenced by tree density in both the years, while fertility levels showed substantial effect only in 2008-09 and by soil depths in both the years (Table 3). The maximum (178.1 kg/ha) more soil available N was recorded with 75% as compared to 50 and 125% of recommended NPK in 2008-09. The differences between 50 and 100; 50 and 125 and 75 with 100% of recommended NPK were not substantial. The available N in soil decreased with increasing soil depths having the maximum (197.3 kg/ha) high soil available N at 0-15 cm depth in both years, comparing to 15-30 and 30-45 cm depths. At 15-30 cm depth higher available N compared to 30-45 cm depth occurred in both years. The maximum available N (kg/ha) was found in surface (0-15 cm) layer during both the years. The lowest available N was recorded in the lower most soil of 30-45 cm.

### *Available Phosphorus in Soil*

The available P in the soil was significantly influenced both by tree density *($P<0.05$)* and fertility levels ($P<0.01$) only in 2008-09, but by soil depths in both the years (Table 3). The maximum P (19.5 kg/ha) was occurred under 1000 trees per ha density compared to other densities including sole crop plots in year 2008-09. The differences amongst sole crop plots and under 250 and 500 trees/ha densities remained statistically non-significant *($P<0.05$)*. The available P in soil increased with increasing fertility levels up to 100% of recommended NPK, while the difference between 75 and 125% of recommended NPK was not significant.

### *Available Potassium (K) in Soil*

The available K in soil after harvest of mustard crop was not influenced by tree density and fertility levels in any of the growing season, but varied with the soil depths in both the years (Table 3). Alike available N and P, available K in soil also decreased with increased soil depths. The maximum (200.9 and 224.5 kg/ha) available K in soil was recorded in 0-15 cm depth which decreased significantly ($P<0.05$) with soil depth both years due to more turn-over of organic residues on the surface soil. The lowest available K was recorded in the lower most soil layer of 30-45 cm.

## Soil Biological Properties

### *Soil Microbial Biomass Carbon*

Soil microbial biomass carbon (SMBC) was significantly ($P<0.05$) influenced by tree density and fertility levels in both the years (Table 4). SMBC increased with high tree densities having maximum (239.0 $\mu g\ g^{-1}$ soil), higher SMBC under highest (i.e. 1000 trees/ha) density both in 2008-09 and 2009-10, respectively, comparing other densities including sole crop in first year and sole crop and 250 trees/ha density in second year. The differences between 250 and 500 trees/ha and 500 and 1000 trees/ha densities were not significant ($P<0.05$) in 2009-10. Among the fertility levels, the maximum 227.88 $\mu g\ g^{-1}$ soil was significantly ($P<0.05$) higher in 75% of

recommended NPK, respectively, compared to 100 and 125% of recommended NPK both in 2008-09 and 2009-10. The differences between 50 and 75% and 100 and 125 % of recommended NPK were not significant (P<0.05) in both the years.

**Table 2.** pH, EC and OC (%) of soil as influenced by poplar tree density, fertility levels and soil depths after harvest of mustard crop during both the growing seasons

| Treatment | pH | | EC (dS/m) | | SOC (%) | |
|---|---|---|---|---|---|---|
| | 2008-09 | 2009-10 | 2008-09 | 2009-10 | 2008-09 | 2009-10 |
| **A. Tree density (trees/ha)** | | | | | | |
| Sole crop | 7.98 | 8.40 | 0.425 | 0.322 | 0.93 | 0.80 |
| 250 | 7.90 | 8.33 | 0.456 | 0.503 | 1.03 | 1.00 |
| 500 | 7.96 | 8.41 | 0.610 | 0.500 | 1.07 | 1.01 |
| 1000 | 7.93 | 8.36 | 0.587 | 0.508 | 1.11 | 1.01 |
| SEm ± | 0.03 | 0.03 | 0.014 | 0.011 | 0.03 | 0.04 |
| CD, 5% | NS | NS | 0.051 | 0.040 | 0.12 | 0.16 |
| **B. Fertility levels (% of recommended NPK)** | | | | | | |
| 50 | 7.93 | 8.36 | 0.678 | 0.466 | 1.02 | 0.96 |
| 75 | 7.93 | 8.38 | 0.431 | 0.452 | 1.11 | 0.91 |
| 100 | 7.96 | 8.39 | 0.468 | 0.480 | 1.00 | 0.98 |
| 125 | 7.96 | 8.37 | 0.500 | 0.434 | 1.01 | 0.97 |
| SEm ± | 0.02 | 0.02 | 0.010 | 0.009 | 0.01 | 0.02 |
| CD, 5% | NS | NS | 0.030 | 0.026 | 0.04 | NS |
| **C. Soil depth (cm)** | | | | | | |
| 0-15 | 7.80 | 8.23 | 0.588 | 0.487 | 1.41 | 1.40 |
| 15-30 | 8.04 | 8.47 | 0.530 | 0.441 | 1.04 | 0.87 |
| 30-45 | 7.98 | 8.42 | 0.441 | 0.447 | 0.68 | 0.60 |
| SEm ± | 0.01 | 0.01 | 0.005 | 0.005 | 0.005 | 0.01 |
| CD, 5% | 0.03 | 0.04 | 0.014 | 0.015 | 0.01 | 0.03 |

NS-Not significant

**Table 3.** Available N, P and K in soil (kg/ha) as influenced by various poplar tree densities, fertility levels and soil depths after harvest of mustard crop during both the growing seasons

| Treatments | Nitrogen | | Phosphorus | | Potassium | |
|---|---|---|---|---|---|---|
| | 2008-09 | 2009-10 | 2008-09 | 2009-10 | 2008-09 | 2009-10 |
| **A. Tree density(trees/ha)** | | | | | | |
| Sole crop | 175.5 | 159.7 | 14.5 | 19.0 | 131.8 | 145.0 |
| 250 | 175.2 | 169.5 | 15.3 | 19.0 | 158.3 | 158.3 |
| 500 | 155.1 | 162.4 | 14.7 | 18.7 | 129.2 | 159.0 |
| 1000 | 155.9 | 163.8 | 19.5 | 18.8 | 143.2 | 151.7 |
| SEm ± | 7.9 | 3.2 | 1.0 | 0.6 | 12.6 | 16.6 |
| CD, 5% | NS | NS | 3.4 | NS | NS | NS |
| **B. Fertility levels(% of recommended NPK)** | | | | | | |
| 50 | 158.4 | 160.9 | 13.2 | 18.0 | 140.5 | 150.4 |
| 75 | 178.1 | 164.3 | 15.8 | 19.6 | 142.5 | 147.0 |
| 100 | 173.6 | 164.3 | 18.4 | 19.7 | 138.7 | 159.4 |
| 125 | 151.5 | 165.9 | 16.6 | 18.3 | 140.8 | 157.2 |
| SEm ± | 5.7 | 1.8 | 0.5 | 0.6 | 4.3 | 6.3 |
| CD, 5% | 16.6 | NS | 1.5 | NS | NS | NS |
| **C. Soil depth (cm)** | | | | | | |
| 0-15 | 197.3 | 191.8 | 26.9 | 27.2 | 200.9 | 224.5 |
| 15-30 | 161.7 | 161.9 | 12.8 | 15.7 | 117.1 | 143.1 |
| 30-45 | 137.2 | 137.9 | 8.3 | 13.8 | 103.9 | 92.9 |
| SEm ± | 3.1 | 1.3 | 0.3 | 0.5 | 3.1 | 3.6 |
| CD, 5% | 8.9 | 3.7 | 1.0 | 1.6 | 8.6 | 10.1 |

NS- Not significant

### *Dehydrogenase Activity*

Dehydrogenase activity was significantly (P<0.05) influenced by tree density during both the years. The dehydrogenase activity was not influenced by fertility levels during both the years (Table 4). Dehydrogenase activity increased with increased tree densities and reached maximum (118 and 167 µg $TPFg^{-1}$ 24 $hr^{-1}$) and significantly(P<0.05) higher under 500 trees/ha density in 2008-09 and 2009-10, respectively, compared to sole crop in first year and sole crop, 250 and 1000 trees/ha densities in second year. The differences between sole crop and 1000 trees/ha and amongst 250, 500 and 1000 trees/ha in 2008-09 and between 250 and 1000 trees/ha densities in 2009-10 were not significant (P<0.05).

## DISCUSSION

The soil bulk density (BD) (g/cc) was substantially influenced by tree density and soil depths in both the years i.e. 2008-09 and 2009-10 (Table 1). The lower bulk density was recorded in higher density due to high organic matter content in closely spaced tree density. More leaf litter fall might occurred in plots under higher tree densities. Higher value of bulk density was observed in second year due to removal of leaf litter in first year from the mustard plot for establishment and proper growth of experimental crop (mustard), hence complete addition of litter to soil could not be allowed. These results are in close agreement with Nayak et al (2009) and Pingale, (2009). Nayak et al (2009) observed the lower bulk density under *Prosopis juliflora* tree as compared to open field. Pingale (2009) also observed decrease in soil bulk density with increased in *Populus deltoides* tree density. The soil bulk density increased with the increase in the soil depth due to poor organic matter content at deeper depth. Gupta and Sharma (2008) also reported soil physical properties such as porosity, density and water holding capacity improved in poplar plantation compared to sole crop in Uttrakhand.

The soil pH varied with soil depths in both the years (Table 2). The marginally lower pH in 0-15 cm soil depth under agroforestry systems may be due to substantial addition of organic matter to the surface soil under trees and organic acid released during litter decomposition as also reported in agrisilvicultural system by Prasadini and Sreemannarayana (2007) and Kumar et al (2008). Newaj et al (2007) also observed very nominal changes in soil pH under White siris (*Albizia procera*) based agrisilvicultural system after 4 years of experimentation as compared to initial value due to very high free calcium carbonate content in the soils. Bharadwaj et al (2001) observed increasing trend of soil pH with deeper soil layers under various tree densities of poplar.

EC of soil underneath poplar trees was higher compared to sole crop (open) in both the years (Table 2). Newaj *et al* (2007) also observed higher EC under *Albizia procera* based agrisilvicultural system than under pure crop of mustard and black gram. This could be due to enrichment of soil mineral basic salts through addition and decomposition of litter. Singh et al (2007) reported that poplar trees returns maximum amount of Ca into the soil through litter fall amongst different macronutrients.

Among the fertility levels, lower (50%) of NPK fertilized plots showed higher value of EC. Maji and Mandal (2004) also observed that addition of 75% of recommended NPK plus FYM either in both the seasons and single season enhanced the electrical conductivity of soil than the control in paddy-oat/berseem rotations. The EC of soil decreased with increasing soil depth. Higher magnitude of EC in 0-15 cm depth compared to lower depth occurred probably due to the presence of more litter at the surface soil. Similar results have also been shown by Newaj et al (2007) in agrisilviculture system in semi-arid areas of India.

Soil organic carbon contents decreased in 2009-10 compared to 2008-09, this might bedue to defoliation of poplar leaves by insect (caterpillar) attack during early autumn season in 2009-10. It is speculated that the defoliation must have reduced the total biomass of organic matter addition into the soil in 2009-10. Such facts might need to be examined in future studies how the caterpillars might impact the biomass loss on soil organic carbon deposits. The SOC (%) in soil increased with increasing the tree densities compared to sole crop i.e. open field. The SOC (%) increased by 11 and 25%; 15 and 26% and 19 and 26% with each successive increase in tree density from zero to 1000/ha in 2008-09 and 2009-10, respectively. Previous studies have also argued that continuous litter addition and tree shading should helped in protecting soil organic matter by reducing oxidation (Gill and Burman 2002, Bharadwaj et al 2001, Pingale 2009). Swami and Puri (2005) also observed 15.1% increase over initial value in SOC (%) in *Gmelina arborea* based agrisilvicultural system, while it has been 51.2% increase in pure plantation of *G. arborea.*

Among the fertility levels, the maximum soil OC was recorded with 75% of recommended NPK compared to other levels of NPK. Das et al (2010) also observed highest soil organic carbon content with lower recommended NPK (50% NPK) in conjunction with *Azolla* compost compared to recommended NPK levels. Moscatelli et al (2008) observed fertilization did not modify soil capacity to accumulate organic matter in the medium term. Organic carbon of soil decreased with increasing soil depth and the maximum SOC was recorded in upper layer compared to deeper layers. Surface soil layer (0-15 cm) showed approximately two times (107 and 133%) more SOC (%) during 2008-09 and 2009-10, respectively. Whereas, the middle soil layer (15-30 cm) contained 53 and 45% more SOC (%) than the lower most layer i.e. 30-45 cm during 2008-09 and 2009-10, respectively. Similar variations in SOC with soil depth have also been indicated by Pingale (2009), Swami and Puri (2005) and Chauhan et al (2010).

The maximum soil available N was recorded with 75% compared to lower (50%) and higher levels (125%) of NPK. This may be due to depressive effect of higher dose of P on nitrogen availability in the soil. Haris et al (1999) had observed the depressed available N status of soils by application of P. The available N, P and K in soil decreased with increased soil depths and the maximum available N, P and K (kg/ha) was found in surface soil (0-15 cm) layer during both the years due to more turn-over of organic residues on the surface soil. The lowest available N, P and K was recorded in the lower most soil layer of 30-45 cm. Bharadwaj et al (2001) also observed decreasing trend in available N, P and K content with increase in soil depth even under high density poplar plantation.

Similarly, Swami et al (2008) also observed available nutrient (NPK) decreased with soil depth under *Gmelina arborea* based agrisilvicultural system. Maximum available P was recorded under highest (1000/ha) tree density probably due to the high organic matter content in the soils. The decomposition of organic matter is accompanied by the release of appreciable quantities of carbon dioxide which forms carbonic acid, when dissolved in water can decompose certain primary minerals. It has been shown that in calcareous soils, carbon dioxide production plays an important role in increasing the phosphate availability. The organic material forms a cover on sesquioxide and reduces the phosphate fixing capacity of the soil (Das et al 2010). The maximum available P in soil was obtained with 100% NPK probably due to the P content of soil increased with increased dose of NPK.

**Table 4.** Soil microbial biomass carbon (μg $g^{-1}$) and dehydrogenase activity (μg TPF $g^{-1}$ $24h^{-1}$) as influenced by poplar tree density and fertility levels at flowering stage during both the growing seasons

| Treatments | Soil microbial biomass carbon | | Dehydrogenase activity | |
|---|---|---|---|---|
| | 2008-09 | 2009-10 | 2008-09 | 2009-10 |
| **A. Tree density(trees/ha)** | | | | |
| Sole crop | 159.00 | 187.00 | 103.00 | 127.00 |
| 250 | 179.00 | 207.00 | 117.00 | 147.00 |
| 500 | 205.00 | 224.00 | 118.00 | 167.00 |
| 1000 | 226.00 | 239.00 | 109.00 | 145.00 |
| SEm ± | 6.04 | 6.01 | 3.49 | 5.09 |
| CD, 5% | 19.31 | 19.22 | 11.15 | 16.28 |
| **B. Fertility levels (% of recommended NPK)** | | | | |
| 50 | 197.73 | 219.70 | 113.03 | 141.80 |
| 75 | 203.10 | 227.88 | 112.98 | 150.70 |
| 100 | 185.41 | 205.29 | 111.39 | 153.40 |
| 125 | 180.82 | 203.35 | 109.79 | 140.60 |
| SEm ± | 3.40 | 3.43 | 1.12 | 4.09 |
| CD, 5% | 9.75 | 9.83 | **NS** | **NS** |

NS=Not significant

Soil microbial biomass carbon increased with increased tree densities owing to higher organic carbon content, and favorable microclimate under higher density. Kumar et al (2008) also observed higher microbial activity under plantations of different tree species in the upper surface of soil due to presence of higher organic carbon content. Chander et al (1995) observed improved microbial biomass carbon during decomposition of poplar leaf litter compared to *Eucalyptus* leaf litter.

Higher SMBC was obtained with 75% of recommended NPK due to adverse effect of higher dose of chemical fertilizers on active microbial population growth. This finding is similar to Cheema et al (2008) where enzymatic activity in soils was found to be depressed with continuous application of recommended inorganic fertilizers. Dehydrogenase activity increased with increased tree densities and reached maximum under 500 trees/ha density. This was probably due to higher fine root biomass under 500/ha tree density. Since the roots are known to produce exudates which are likely to provide energy for microbial metabolic activity. Singh and Singh (1994) also observed fine roots biomass of poplar was more in wider spacing, generally in 0-15 cm soil layers. The dehydrogenase activity improved underneath trees comparing to sole crop in both the years indicating increased active microbial population, as it provides correlative information on the biological activity and microbial population in the soil. This improvement in dehydrogenase activity might be due to improved physico-chemical properties of soil by trees. Batra (2004) also observed the dehydrogenase activity of soil being influenced both by the soil physico-chemical characteristics and agricultural management practices.

Agroforestry land use system is viable for sustainable agricultural production due to substantial addition of organic matter into the soil. The soil quality in enhanced underneath trees and optimum tree density is required for proper crop growth under tree canopy. Optimum dose of NPK (75%) is required for maintaining the soil quality in agrisilviculture system.

Agriculture land is believed to be a major potential sink and could absorb large quantities of carbon, if trees are reintroduced to these systems and judiciously managed together with the crop and/or animals. Thus importance of agroforestry as a land use system is receiving a wider recognition not only in terms of agricultural sustainability, but also due to climate change. Litter fall plays an important role in terms of nutrient cycling, and thus adds to sustainability of the system.

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# Wheat Yield Trend and Soil Fertility Status in Long Term Rice-Rice-Wheat Cropping System

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## ABSTRACT

A long-term soil fertility experiment under rice-rice-wheat system was performed to evaluate the long term effects of inorganic fertilizer and manure applications on soil properties and grain yield of wheat. The experiment began since 1978 was laid out in randomized complete block design with 9 treatments replicated 3 times. From 1990 onwards, periodic modifications have been made in all the treatments splitting the plots in two equal halves of 4 x 3 $m^2$ leaving one half as original. In the original treatments, recent data revealed that the use of Farm Yard Manure (FYM) @10 t $ha^{-1}$ gave significantly ($P \leq 0.05$) higher yield of 2.3 t $ha^{-1}$ in wheat, whereas control plot gave the lowest grain yield of 277 kg $ha^{-1}$. Similarly, in the modified treatments, the use of FYM @10 t $ha^{-1}$ along with inorganic Nitrogen (N) and Potassium oxide ($K_2O$) @ 50 kg $ha^{-1}$ produced significantly ($P \leq 0.05$) the highest yield of 2.4 t/ha in wheat. The control plot with an indigenous nutrient supply only produced wheat yield of 277 kg $ha^{-1}$ after 35th year completion of rice-rice-wheat system. A sharp decline in wheat yields was noted in minus N, phosphorus (P), Potassium (K) treatments during recent years. Yields were consistently higher in the N:$P_2O_5$:$K_2O$ and FYM treatments than in treatments, where one or more nutrients were lacking. The application of $P_2O_5$ and $K_2O$ caused a partial recovery of yield in P and K deficient plots. There was significant ($P \leq 0.05$) effect of use of chemical fertilizers and manure on soil properties. The soil analysis data showed an improvement in soil pH (7.8), soil organic matter (4.1%), total N content (0.16%), available P (503.5 kg $P_2O_5$ $ha^{-1}$) and exchangeable K (137.5 kg $K_2O$ $ha^{-1}$) in FYM applied treatments over all other treatments. The findings showed that the productivity of the wheat can be increased and sustained by improving nutrient through the integrated use of organic and inorganic manures in long term.

**Key words**: FYM, inorganic, long term, organic and rice-rice-wheat

## सारांश

धान-धान-गहुँ वाली प्रणालीमा रासायनिक र प्राङ्गारिक मलको वाली उत्पादन र माटोको उर्वरकतामा पर्न सक्ने दिर्घकालिन असरको शोधकार्य लागी सन् १९७८ देखि राष्ट्रिय गहुँ वाली अनुसन्धान कार्यक्रम भैरहवामा एक परीक्षण शुरु गरिएको थियो । उक्त परीक्षण रेन्डोमाईज्ड कम्पीलीट ब्लक डिजाईनमा तीन पटक छुट्टाछुट्टै गरिएको थियो । सन् १९९० पछि समय अनुसार उपचारहरुलाई दुई बराबर भागमा विभाजन गरिदै लगियो । जस अनुसार ४×३ बर्ग मिटरको दुई बराबर भागमा आधी भागलाई मूल उपचारको रुपमा राखियो । मूल उपचारहरुको हालको आंकडाले गोठेमलको १० टन प्रति हेक्टरको योगवाट गहुँको उत्पादन २.३ टन प्रति हेक्टरको पाईयो भने, कुनै पनि मल नराखेको उपचारमा सबै भन्दा कम २७७ के जी प्रति हेक्टर उत्पादन भयो । त्यसैगरी, परिमार्जित उपचारमा तत्कालको आंकडा अनुसार गोठेमल १० टन प्रति हेक्टर र रासायनिक मल ५० के जी नाईट्रोजन र ५० के जी पोटासियम प्रतिहेक्टरको संयुक्त प्रयोगले गहुँको २.४ टन प्रति हेक्टर उल्लेखनिय बढी उत्पादन भएको पाइयो । धान-धान-गहुँ प्रणालीमा माटोको पोषक आपूर्तिले नियन्त्रित गहुँमा ३५ औं बर्ष पछि २७७ के.जी. प्रति हेक्टर मात्र गहुँको उत्पादन भयो । हालका बर्षहरुमा नाईट्रोजन, फस्फोरस र पोटासियम नराखेको उपचारमा गहुँको उत्पादनमा धेरैनै कमी आयो । एन. पि. के. र गोठेमल राखेको उपचारहरुमा कुनै एक वा एक भन्दा बढी पोषकतत्व नराखेको उपचारमा भन्दा गहुँको उत्पादन निकै बढी थियो । फस्फोरस र पोटासियमको प्रयोगबाट गहुँको उत्पादनमा कमी आएको देखियो । माटोको गुणमा रासायनिक मलको प्रयोग र खादको महत्वपूर्ण प्रभाव थियो । माटोको विश्लेषण आंकडा अनुसार अन्य सबै उपचार संग तुलना गर्दा गोठेमलको प्रयोग गरिएको प्लटहरुमा माटोको पी एच ७.८, प्राङ्गारिक पदार्थ ४.१%, कुल नाईट्रोजन ०.१६, उपलब्ध फस्फोरस ५०३.५ र पोटासियम १३७.५ किलो प्रतिहेक्टर रहि सुधारात्मक रहेको पाइयो । नेपालको परिप्रेक्षमा प्राङ्गारिक र रासायनिक मलको दिर्घकालिन एकीकृत उपयोगबाट गहुँको उत्पादकत्वमा अभिबृद्धि हुनुका साथै लामो समयको लागि पोषकतत्वमा सुधार हुन् सक्ने निष्कर्ष रहेको देखिन्छ ।

## INTRODUCTION

Rice and wheat mostly grown in sequencing order especially in Terai region represents the most important food crops of Nepal. The Terai plain, meets about 75% of the country's total cereal food demand (NARC 1995). However, productivity and profitability are quite low despite of the fact that a doubling of the crop production in the next 25 years is required to meet Nepal's estimated population growth (NARC 1995, Hobbs and Morris 1996, Kshteri 2010). Increasing the productivity of land through intensive cropping system depletes nutrient reserves of the soil at faster rate (Regmi et al 2003) while unbalanced fertilizer application may disturb nutrient availability to crops, leading to a reduction in yield (Nambiar 1994, Yadav et al 2000). Improving productivity and increasing cropping intensity to sustain yields and meet the future food needs requires adequate soil fertility (Regmi et al 2003). Therefore, to maintain land productivity for crop production, it is likely that new emerging nutrient deficiency or imbalances are addressed, identified and corrected promptly. In such sequence, identification of micronutrients zinc deficiency in rice and boron in wheat represents the best examples (Regmi et al 2003).

It has been observed that continuous use of chemical fertilizers in imbalanced form deteriorates soil physical properties (Biswas et al 1971, Prasad et al 1983, Bhattacharyya et al 2015). Detrimental effects of chemical fertilizers even in balanced form on soil physical properties are also being observed. For example, decline in soil organic carbon and associated decline in system productivity under rice–wheat system with the long-term use of recommended NPK was observed in some field studies (Nambiar 1994, Abroal et al 2000, Yadav et al 2000). The widespread stagnation and occasional decline is rice–wheat productivity over the last about three decades have become a matter of serious concern, as rice–wheat is the major cropping system in south Asia, feeding more than 400 million people world-over (Ladha et al 2003).

Soil fertility is largely attempted to maintain by application of compost and manure, but in recent years a decline in soil fertility has been reported (Shrestha et al 2000). There has been considerable research in Nepal on soil fertility enhancement and soil and water conservation techniques over the years (Keatinge et al 1999, Acharya et al 2000). Despite of that the soil fertility in long run in relation to cropping pattern has always been a subject of interest because of several factors associated including the manure and fertilizer use. Therefore, long-term experiment has always been suggested to be valuable for evaluating the effects of continuous cropping on the capacity of a system to sustain nutrient supply and the productivity (Regmi et al 2003).

For recommending appropriate technologies to maintain soil fertility as well as the environment, it is necessary to evaluate long-term effects of inorganic and organic manures on soil properties (Gami and Sah 1988, Bhattacharya et al 2015). The long-term soil fertility experiments are valuable for understanding the relationships among changing soil, crop management practices and productivity (Bhattacharya et al 2015). It is also important that the data collected from constantly monitoring long-term experiment could be useful for improving statistical and simulation tools (IRRI 2000, Bhattachrayya 2015). Therefore, a long term experiment was initiated under rice-rice-wheat system to evaluate the effects of long term application of mineral fertilizer and organic manure on soil properties and wheat grain yield under rice-rice-wheat system.

## MATERIALS AND METHODS

### Experimental Site, Treatments and Crop Management

The long term experiment was started in 1979/80 at National Wheat Research Program located in Bhairahawa, Rupandehi District in the western Terai Region of Nepal at the latitude of 27°32' and the longitude of 83°28' with an elevation of 120 masl. In this site, the air temperature ranges from a minimum of about 7°C in winter to the maximum of about 45$^0$C in summer. In general, the site receives ample rainfall during the monsoon, which starts from June and continues up to September. The mean annual rainfall is about 1800 mm. The soil of the experiment plot was silty loam with a pH of 8.0, organic matter (OM) of 1.783%, P of 9.75 u/g, exchangeable K of 126 ug/g soil and bulk density of 1.6 g cm$^{-3}$ with hard pan just below the plow layer. The soils in the experiment area are classified as Typic Heplaquepts.

The experiment was laid out in randomized complete block design with 9 treatments which were replicated 3 times. The plot size was 6 x 4 m$^2$ up to 1990. From 1990 onwards, periodic modifications have been made in all the treatments splitting the plots in two equal halves of 4 x 3 m$^2$ leaving one half as original (Table 1). Wheat was sown in rows of 25 cm apart. Farm yard manure was applied at 7-10 days before seeding. Half dose of N and full dose of P and K were applied as basal. Remaining 50% nitrogen was top dressed at 21-25 days after seeding in wheat.

### Measurement of Crop Parameters

**Table 1.** Original and modified treatments of LTSFE (R-R-W)

| Tr No | Original Treatment N, $P_2O_5$, $K_2O$ kg/ha | Modified Tr. (1991) N, $P_2O_5$, $K_2O$ kg/ha | Modified Tr.(1995 onward) N, $P_2O_5$, $K_2O$ kg/ha |
|---|---|---|---|
| 1 | 0: 0:0 - R & W | 0: 0:0 - R & W | 100:50:100- R & W |
| 2 | 100:0:0- R & W | 100:30:30 - R<br>100:40:30- W | 100:30:30 - R<br>100:40:30- W |
| 3 | 100:30:0 - R<br>100:40:0 – W | 100:30:0 - R<br>100:40:0 - W | 100:30:30- R<br>100:40:30 - W |
| 4 | 100:0:30- R & W | 100:100:30<br>One time - ER | 100:30:30 – R<br>100:40:30 - W |
| 5 | 100:30:30 – R<br>100:40:30 – W | 100:30:30 – R<br>100:40:30 - W | 100:30:100 – R<br>100:40:100 - W |
| 6 | 100:0:0 – R<br>100:40:30 – W | 100:30:0 - ER | 100:0:0 – R<br>100:40:30 – W<br>100:30:0 - ER |
| 7 | 50:0:0 - R & W+ 30 cm stubble incorporation | 50:20:0 - R & W +30 cm stb. incorporation | 50:20:0 - R & W +30 cm stb. incorporation |
| 8 | 50:20:0 - R & W+ 30 cm stubble incorporation | 50:20:0 - R & W+ 30 cm stubble incorporation | 50:20:20 - R & W + 30 cm stubble incorporation |
| 9 | F Y M 10 t/ha - R & W | F Y M 10 t/ha + 50 kg N - R & W | F Y M 10 t/ha + 50 kg N + 50 kg K20 - R & W |

LTSFE= Long Term Soil Fertility Trial, Trt= Treatments

### Soil Sampling and Analysis

Soil samples were collected from each of the experimental plots after harvesting wheat (5 April 2014). Each soil sample was randomly collected from the 0 to 20 cm deep plough layer using an auger. For this, the air-dried samples were crushed and passed though a 2 mm sieve. Soil pH was determined by a pH meter after extraction from a soil: water ratio of 1:2. Organic matter was determined using the Walkley and Black dichromate method (Nelson and Sommers 1982) and total N using Kjeldhal's method (Bremner and Mulvaney 1982) For available P determination modified Olsen's (Olson and Sommers 1982 ) methods used; exchangeable K was estimated by 1M ammonium acetate extraction (Knudsen et al 1982) followed by flame photometric determination.

## Statistical Analysis

Recorded data were compiled and tabulated in Ms-Excel. Data for each parameter over two year's period was subjected to analysis of variance using a Randomized Complete Block Design according to MSTATC (Steel and Torrie 1980). Treatment means were compared using least significant difference (LSD) test at $P \leq 0.05$.

## RESULTS

Grain yields of wheat in rice-rice-wheat system were affected by the application of different combination of manures and fertilizer treatments.

## Original Treatments

The various growth and yield attributing parameters significantly ($P \leq 0.05$) differed with the varied use of organic and inorganic sources of nutrients in wheat crop. The plant height, productive tillers per $m^2$, test weight, straw and grain yields were significantly ($P \leq 0.05$) higher where use of FYM @10 t $ha^{-1}$ were applied with the highest yield of 3616 kg $ha^{-1}$ in 2011 and 2383 kg $ha^{-1}$ in 2012 against 425 kg $ha^{-1}$ and 277 kg $ha^{-1}$ in control (T1), respectively (Appendix 1, 2 and 3). In 2012/13, logging during heading stage of wheat caused the low yield.

**Table 2.** Grain yield (kg $ha^{-1}$) of wheat in LTFT during 2011/12 and 2012/13

| Trt | 2011/12 | | 2012/13 | |
|---|---|---|---|---|
| | Original | Modified | Original | Modified |
| T1 | 425 | 3278 | 277 | 1793 |
| T2 | 1218 | 1865 | 370 | 1521 |
| T3 | 1582 | 2124 | 333 | 1538 |
| T4 | 513 | 3176 | 370 | 1682 |
| T5 | 2622 | 3348 | 1325 | 1899 |
| T6 | 2612 | 2808 | 1205 | 1418 |
| T7 | 386 | 1695 | 362 | 1085 |
| T8 | 1707 | 2524 | 1064 | 1709 |
| T9 | 3616 | 3799 | 2278 | 2383 |
| F test | *** | ** | *** | * |
| CV, % | 25.9 | 21.5 | 16.6 | 16.4 |
| LSD (0.05) | 1296 | 1015.8 | 223.5 | 462.2 |
| F test | *** | ** | *** | * |

LTFT= Long Term Fertility Trial, Trt= Treatments

## Modified Treatments

The highest grain yield (3799 kg $ha^{-1}$ in 2011/12 and 2383 kg $ha^{-1}$ in 2012/13) was occurred in treatment-T9 ie FYM @10 t $ha^{-1}$ + N @50 kg + 50 kg $K_20$, where as application of N: $P_2O_5$: $K_2O$ @50:20:0 plus 30 cm stubble incorporation gave the lowest grain yield of wheat (1695 kg $ha^{-1}$ in 2011/12 and 1085 kg $ha^{-1}$ in 2012/12) probably due to absence of K in the treatment (Table 2). This alarms situation in farmers' field where farmers generally do not apply potassium fertilizer in wheat crop.

## Yield Trend of Wheat

The data revealed that the grain yield of wheat was higher in treatment T9M (FYM 10 t $ha^{-1}$ and N and $K_2O$ 50 kg $ha^{-1}$ each which is followed by FYM application alone (T9) and the recommended fertilizer dose (T5). The results showed that yield trends of wheat for N treatment (T2), NK treatment (T4) and NP treatment (T3) were similar to the control (no-fertilizer) by end of 35 years of the experiment (Figure 1, 2 and 3).

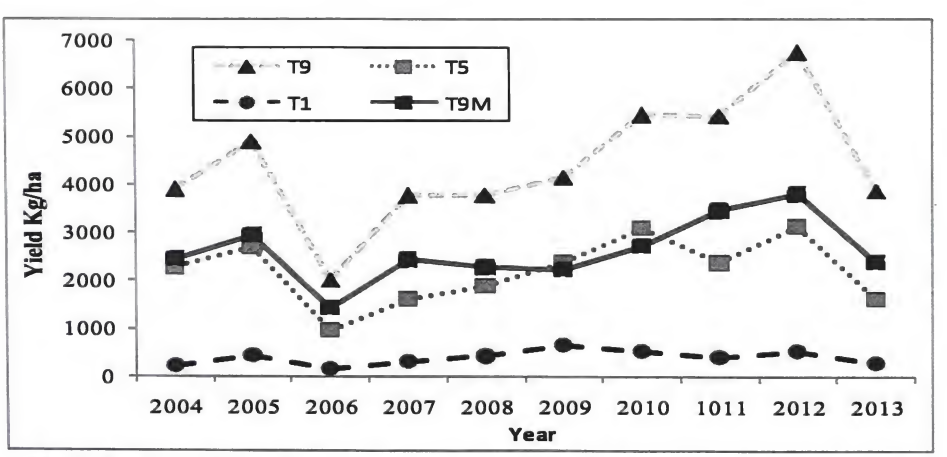


**Figure 1.** Grain yield trend of long-term application of fertilizers and organic manure on wheat.

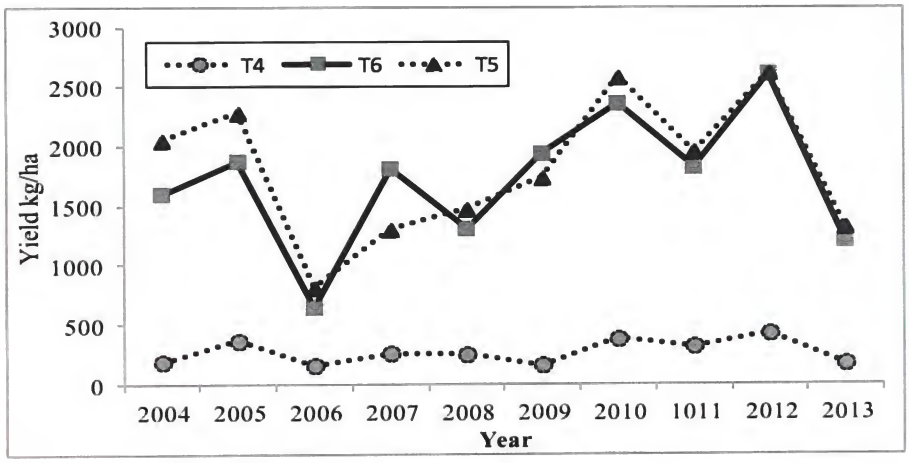


**Figure 2.** Effect of P addition on grain yield of wheat.

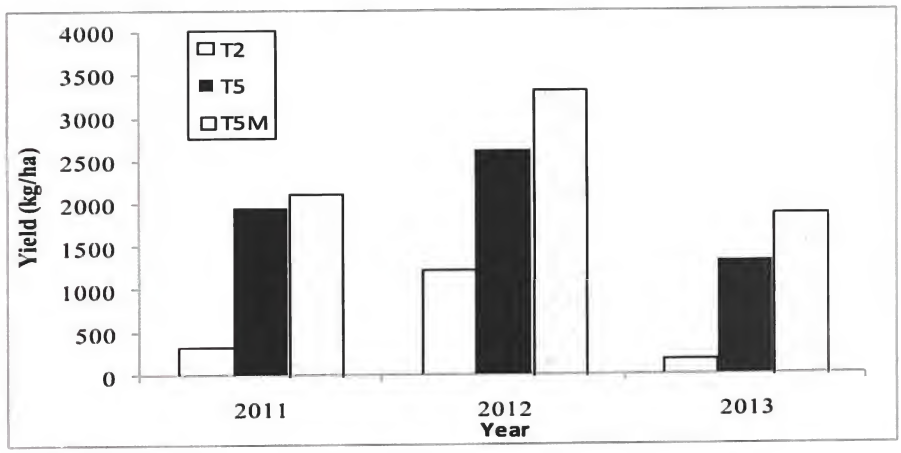


**Figure 3.** Effect of P and K omission on grain yield of wheat.

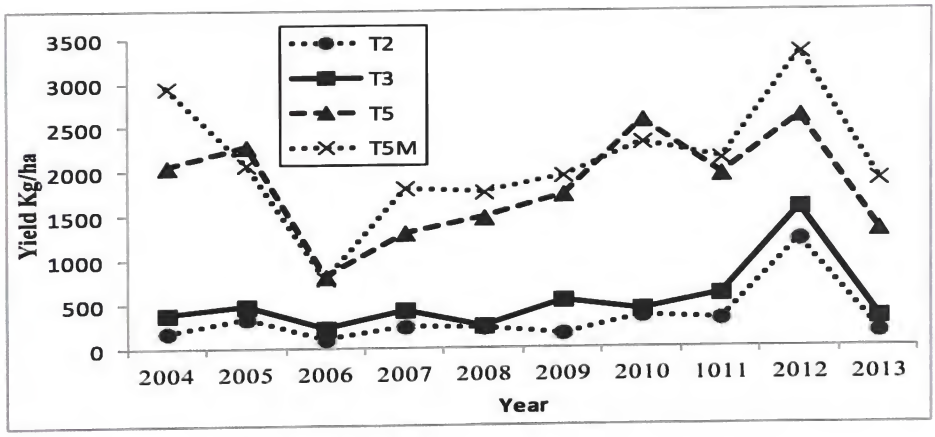


**Figure 4.** Addition of K increases wheat yield.

## Soil Fertility Status

There was a significant ($P \leq 0.05$) effect of manures and fertilizers on soil pH, soil organic matter, N content, available P and exchangeable K. At harvest of wheat crop, soil pH was improved in T9 (7.87) from the application of farm yard manure as compared to original (8.0) and significantly ($P \leq 0.05$) higher SOM (4.12%) was obtained by applying the FYM and inorganic fertilizer which was statistically similar with the application of FYM alone (4.10%) (Table 3). Similarly, significantly ($P \leq 0.05$) higher soil N content was

obtained from the combined application of FYM (0.16%) but it was similar to the application of FYM plus chemical fertilizer (N and $K_2O$) and it was the lowest (1.13%) in control plot (0.8 %).

At the end of 35 years experiment, the highest soil available P of 503.5 kg $ha^{-1}$ was occurred in the plots applied with FYM, while the lowest available soil P of 11.5 kg $ha^{-1}$ was in the control plot. Similarly, exchangeable K was significantly ($P \leq 0.05$) higher (137.8 kg $ha^{-1}$) with N and $K_2O$ which was at par with the application of FYM alone (117.1 kg $ha^{-1}$). The soil analysis data showed an improvement in soil pH (7.8), soil organic matter (4.1%), total nitrogen content (0.16%), available P (503.5 $P_2O_5$ kg $ha^{-1}$) and exchangeable K (117.1 $K_2O$ kg $ha^{-1}$) in FYM applied treatments over all other treatments.

**Table 3.** Soil chemical properties affected by organic & chemical fertilizers NWRP, Bhairahawa, Nepal

| Treatment | pH | | Organic matter (%) | | Nitrogen (%) | | $P_2O_5$ (kg $ha^{-1}$) | | $K_2O$ (kg $ha^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Original | Modified | Original | Modified | Original | Modified | Original | Modified | Original | Modified |
| $T_1$ | 8.25 | 8.11 | 1.27 | 1.89 | 0.08 | 0.09 | 11.5 | 123.8 | 94.3 | 117.1 |
| $T_2$ | 8.25 | 8.18 | 1.67 | 2.18 | 0.09 | 0.10 | 11.7 | 50.0 | 76.0 | 80.6 |
| $T_3$ | 8.16 | 8.17 | 2.15 | 2.33 | 0.10 | 0.11 | 93.7 | 70.5 | 34.9 | 76.0 |
| $T_4$ | 8.23 | 8.14 | 1.67 | 1.89 | 0.09 | 0.09 | 14.7 | 40.8 | 103.4 | 53.2 |
| $T_5$ | 8.21 | 8.16 | 2.30 | 2.39 | 0.11 | 0.11 | 44.4 | 57.2 | 71.5 | 103.4 |
| $T_6$ | 8.20 | 8.21 | 2.15 | 1.65 | 0.10 | 0.09 | 10.7 | 34.0 | 62.3 | 62.3 |
| $T_7$ | 8.23 | 8.17 | 2.02 | 2.13 | 0.10 | 0.10 | 12.3 | 28.8 | 76.0 | 66.9 |
| $T_8$ | 8.18 | 8.15 | 2.06 | 1.86 | 0.10 | 0.93 | 38 | 34.4 | 71.5 | 76.0 |
| $T_9$ | 7.87 | 7.95 | 4.10 | 4.12 | 0.16 | 0.16 | 503.5 | 403.7 | 117.1 | 137.8 |
| F-test | ** | Ns | *** | ** | *** | ** | *** | *** | * | * |
| LSD (0.05) | 0.16 | - | 0.89 | 0.99 | 0.03 | 0.03 | 45.63 | 49.86 | 40.57 | 40.67 |
| CV, % | 1.1 | 1.1 | 23.9 | 25.2 | 14.7 | 15.8 | 32.1 | 30.8 | 29.8 | 28.4 |
| Initial (1978/79 AD) | 8.0 | | 1.025 | | 0.088 | | 9.8 | | 126 | |

***, ** and * denotes significant at 0.1%, 1% and 5% level of significance respectively and Ns stands for non significant
LTFT= Long Term Fertility Trial

## DISCUSSION

Soil fertility and plant nutrient management are key issues to be addressed to understand the reasons for declining crop yields. After 35 years of the experiment, significantly ($P \leq 0.05$) higher yield of wheat was found in T5, T9 and T9M as compared to other treatments (Figure 1). There was fluctuation in grain yield of wheat which could be due to variation in rainfall, temperature, moisture in general during crop growing period. There was very low grain yield in all P missing treatments (T1, T2, T4, and T7) (Figure 1, 2 and 3). This shows P is one of the most limiting factors for wheat crop. In all treatments in which one or more primary nutrients were lacking, resulted decline in wheat grain yield. This finding was similar to many other studies earlier reported (Dawe and Dobermann 1999, Hobbs and Morris 1996). These implying either the changes in biochemical and physical composition of soil organic matter (SOM) might resulted in gradual decline in the supply of soil nutrients to crop due to inappropriate fertilizer applications causing nutrient imbalances (macro and micro) (Paroda et al 1994).

The grain yield of wheat was found higher in T5 (100:30:30 – R and 100:40:30 – W) followed by T6 (100:0:0 – R and 100:40:30 - W). The significant ($P \leq 0.05$) differences in grain yield were seen between the N: $P_2O_5$: $K_2O$ and N: $P_2O_5$ treatments, indicating that the yield reductions in this experiment were also due to the K deficiency. With the increase in K level, there was increase in the grain yield of normal wheat (Figure 4).

Proper use of chemical fertilizers and organic manures supports increased agricultural productivity and at the same time, helps maintain soil fertility (Gami and Sah 1988). Similar results have been revealed in present study. The results showed that neither the present dose of N: $P_2O_5$: $K_2O$ nor FYM can sustain productivity in such system. These results corroborate those of Flinn and De Datta (1984), who reported a yield decline under the full recommended dose of fertilizer. In many fertilizer experiments, Nambiar and Abrol (1989) have also found a declining trend with adequate NPK. FYM alone could not supply N and K requirement of wheat crop. The yield increase with the modified (T9M) possibly resulted from replacement of the original (T9) due to balance dose of N: $P_2O_5$: $K_2O$ and other micronutrients contained in FYM.

In the long run, soil fertility is not sustained from the balanced fertilizer application alone while application of FYM helps to boost up crop yield (Lal and Mathur 1989, Kabeerthumma et al 1993) and improve physical soil status (Lal and Mathur 1989b, Kumar and Tripathi 1990). Declining yield trend at lower N level over the years may indicate the diminishing supply capacity of soil. The single greatest cause of declining crop production is unbalanced fertilization (Rattan and Singh 1997). Unbalanced fertilizer application has led to a chronological emergence of macronutrients such as phosphorus and potash (P and K) and micronutrients such as zinc, sulfur and manganese (Zn, S and Mn) deficiencies. Even balanced application of macronutrients devoid of organic materials has been implicated in the deterioration of the physical, chemical and biological health of soil (Rattan and Singh 1997).

Our findings also showed that the regular application of FYM might significantly increase the available phosphorus, total nitrogen and carbon contents in the soil but not the potassium level (Table 3). The five-year long-term experiment clear showed that the recommended chemical fertilizer dose of 100:40:30 (N: $P_2O_5$: $K_2O$) kg $ha^{-1}$ for wheat crop seems to be inadequate for boosting yield and soil productivity. Long-term application of FYM @ 10-tons $ha^{-1}$ was found to enhance its yield and increased the soil nutrients content in the soil. Balanced application is considered a sustainable way of enriching soil and hence restoring its fertility over time (Bhattachryya et al 2015). Similar long term fertility experiments showed that depletion of soil nutrients caused by years of intensive cropping is a common feature that appears to contribute to the observed yield decline (Regmi 1996, Yadvinder-Singh et al 2000). In

addition to mining of nutrients, there is some evidence of changes in the availability of nutrients to the plant. In most rice-wheat experiments, the soil organic matter declines over time, but, it is not only the decline per season; there may also be changes in the chemical composition of organic matter (Olk et al 1996, 1998, Bronson and Hobbs 1998) that influence the capacity to supply nutrients to the plant. A sustainable fertilizer management strategy must ensure high and stable overall productivity and sufficient nutrient supply for potential yield increases.

The control plot with an indigenous nutrient supply only supported wheat yield of 277 kg $ha^{-1}$ in long term experiments of rice-rice-wheat system (Figure 1). FYM alone could not supply N and K requirement of wheat crop. The increase in wheat yield was observed due to the application of 50 kg $ha^{-1}$ both N and $K_2O$ to FYM treatment. The lowest grain yield of wheat may be due to absence of potassium in the treatment. This alarms situation in farmers' field where farmers generally do not apply potassium fertilizer in wheat crop. The soil analysis data showed an improvement in soil pH, soil organic matter, total nitrogen content, available phosphorus and exchangeable potassium in FYM applied treatments over all other treatments. Declining yields and soil nutrient balance in a long-term rice-rice-wheat study suggest depletion of soil potassium (K) and P fertilization seem to be primary reasons for limited and declining crop yields. The findings showed that the productivity of the wheat can be increased and sustained by improving nutrient through the integrated use of organic and inorganic manures in long term.

## ACKNOWLEDGMENTS

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## APPENDIXES

**Appendix 1.** Rating chart of soil values to determine the fertility status of experimental soil

| Nutrient | Low | Medium | High |
|---|---|---|---|
| Available N (%) | <0.10 | 0.1-0.2 | >0.2 |
| Available $P_2O_5$ (kg/ha) | <30 | 30-55 | >55 |
| Available $K_2O$ (kg/ha) | <110 | 110-280 | >280 |
| Organic matter (%) | <2.5 | 2.5-5.0 | >5.0 |
| pH | <6.0 (Acidic) | 6.0-7.5 (Neutral) | >7.5 (Alkaline) |

Source: Khatri Chettri 1991 and Jaishy 2000

**Appendix 2.** Plant height, productive tillers/$m^2$, 1000-grain weight, straw yield and grain yield of wheat in LTFT conducted at NWRP, Bhairahawa, 2011/12

| Trt | Plant height, cm | | Tillers/$m^2$ (n) | | 1000-grain weight (g) | | Straw yield (kg $ha^{-1}$) | | Grain yield (kg $ha^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Original | Modified | Original | Modified | Original | Modified | Original | Modified | Original | Modified |
| $T_1$ | 66.33 | 100.87 | 187.7 | 220.3 | 30.53 | 42.40 | 1722 | 7611 | 425 | 3278 |
| $T_2$ | 59.87 | 93.20 | 167.3 | 225.0 | 23.07 | 38.75 | 1389 | 6778 | 1218 | 1865 |
| $T_3$ | 91.77 | 87.80 | 237.3 | 214.3 | 35.73 | 32.73 | 6389 | 5222 | 1582 | 2124 |
| $T_4$ | 64.90 | 96.47 | 206.3 | 255.3 | 29.20 | 37.07 | 1722 | 7389 | 513 | 3176 |
| $T_5$ | 94.70 | 99.47 | 229.7 | 234.3 | 36.87 | 41.57 | 6389 | 7389 | 2622 | 3348 |
| $T_6$ | 94.13 | 95.10 | 237.3 | 230.0 | 40.40 | 38.13 | 6222 | 6444 | 2612 | 2808 |
| $T_7$ | 60.90 | 83.03 | 163.3 | 221.3 | 26.33 | 35.47 | 1278 | 3444 | 386 | 1695 |
| $T_8$ | 81.87 | 92.37 | 199.0 | 230.7 | 31.00 | 41.07 | 3500 | 4778 | 1707 | 2524 |
| $T_9$ | 97.53 | 100.03 | 228.0 | 239.7 | 44.07 | 45.33 | 8167 | 8833 | 3616 | 3799 |
| F test | *** | ** | ** | Ns | *** | ** | *** | *** | *** | ** |
| CV, | 4.4 | 5.0 | 10.8 | 12.1 | 7.0 | 8.1 | 14.1 | 13.6 | 25.9 | 21.5 |
| LSD | 6.032 | 8.237 | 38.71 | 48.36 | 3.986 | 5.519 | 997.4 | 1510.4 | 1296 | 1015.8 |

***, ** and * denotes significant at 0.1%, 1% and 5% level of significance respectively and Ns stands for non significant
LTFT= Long Term Fertility Trial, GY= Grain Yield, Trt= Treatments

**Appendix 3.** Plant height, productive tillers/$m^2$ test weight, straw yield and grain yield of wheat per hectare in LTFT conducted at NWRP, Bhairahawa, 2012/13

| Trt | PH | | spikes/$m^2$ | | 1000-grain wt. | | Biomass | | Grain yield (kg $ha^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Original | Modified | Original | Modified | Original | Modified | Original | Modified | Original | Modified |
| $T_1$ | 65.00 | 91.33 | 144 | 176.7 | 28.81 | 32.48 | 1111 | 4389 | 277 | 1793 |
| $T_2$ | 58.00 | 83.67 | 163 | 207.3 | 25.27 | 26.29 | 889 | 4000 | 370 | 1521 |
| $T_3$ | 66.67 | 84.44 | 134 | 196.0 | 19.81 | 27.36 | 1278 | 4278 | 333 | 1538 |
| $T_4$ | 59.67 | 83.67 | 130 | 180.0 | 30.14 | 28.23 | 1000 | 43.89 | 370 | 1682 |
| $T_5$ | 82.67 | 91.67 | 205 | 179.7 | 31.00 | 30.17 | 3778 | 6944 | 1325 | 1899 |
| $T_6$ | 83.33 | 82.00 | 171 | 161.7 | 27.79 | 27.42 | 3444 | 3278 | 1205 | 1418 |
| $T_7$ | 55.67 | 77.00 | 132 | 183.3 | 27.19 | 25.99 | 889 | 2878 | 362 | 1085 |
| $T_8$ | 84.67 | 89.67 | 156 | 221.3 | 27.07 | 31.92 | 2556 | 3711 | 1064 | 1709 |
| $T_9$ | 90.00 | 95.67 | 215 | 220.3 | 38.19 | 30.77 | 6000 | 6600 | 2278 | 2383 |
| F-test | *** | ** | ** | Ns | *** | Ns | *** | Ns | *** | * |
| CV, % | 4.4 | 3.1 | 16.3 | 17.0 | 7.2 | 9.2 | 11.5 | 33.9 | 16.6 | 16.4 |
| LSD | 5.41 | 4.64 | 45.51 | 56.40 | 3.55 | 4.63 | 462.0 | 3351.7 | 223.5 | 462.2 |

***, ** and * denotes significant at 0.1%, 1% and 5% level of significance respectively and Ns stands for non significant
LTFT= Long Term Fertility Trial, Trt= Treatments

# Management of Anthracnose in Soybean using Fungicide

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## ABSTRACT

Experiments on soybean (*Glycine max* L. Meril) were carried out aiming to control anthracnose (pod blight) caused by fungus, *Colletotrichum truncatum* with five treatments represented by different fungicidal sprays against control receiving no spray with three replicates of each under field conditions during two consecutive years from 2012 to 2013. In 2012, the higher Percent Disease Control (PDC) and Percent Yield Increase (PYI) were estimated in plot treated with SAAF (Carbendazim 12% + Mancozeb 63%) followed by Mancozeb fungicides. The mean Pod Infection (PI) was low in plots treated with SAAF followed by Mancozeb. Almost similar trends of disease control were observed in 2013. The lower Percent Disease Index (PDI) was 46.25% and mean PI was 29.67% with higher yield value of 2431.25 kg/ha obtained from the plots sprayed with SAAF then by Mancozeb. The results showed that, the combined treatment with fungicides, SAAF followed by Mancozeb were effective to control anthracnose or pod blight disease of soybean to increase the yield.

**Key words:** Anthracnose, fungicides, pod blight, soybean

## सारांश

भटमास बालीमा कोलेटोट्रीकम ट्रन्काटम नामक ढुँसीले लाग्ने कोसा डढ्ने रोग व्यवस्थापन गर्ने उद्देश्यले रामपुर चितवनमा सन् २०१२ देखि र २०१३ मा बजारमा उपलब्ध विभिन्न ढुँसीनाशक विषादीहरुको रोग नियन्त्रण प्रभावकारिता परीक्षण गरिएको थियो । सन् २०१२ को वर्षायाममा ढुँसीनाशक विषादी साफ (कारबेन्डाजीम १२% + म्यान्कोजेब ६३%) बाट उपचारित प्लटहरुमा रोग नियन्त्रण प्रभावकारिता ४२.१९% र उत्पादनमा वृदि १०८.६९% रहेको र त्यसपछि म्यान्कोजेबद्वारा उपचारित प्लटमा पनि केही पनि नछरेको प्लटभन्दा उच्च रहेको पाइयो । कोसामा देखिने रोगको संक्रमणको औषत प्रतिशत पनि क्रमशः साफ र म्यान्कोजेब द्वारा उपचारित प्लटमा क्रमसः कम (२२.३१% र २३.०४%) रहेको पाइयो । सन् २०१३ को परिक्षणमा पनि अघिल्लो वर्षको रोग व्यवस्थापनको आंकडा जस्तै नतिजा प्राप्त भयो । सबैभन्दा कम रोग र कोसामा रोगको संक्रमणको औषत प्रतिशत साथै उच्च उत्पादन क्रमशः ४६.२५%, २९.६७% र २४३१.२५ के.जी. प्रति हेक्टर साफ द्वारा उपचारित प्लटमा पाइयो भने, त्यसपछि म्यान्कोजेब द्वारा उपचारित प्लटमा थियो । यसर्थ, दुई वर्षको परिक्षणको नतिजा अनुसार ढुँसीनाशक विषादीहरु साफ र म्यान्कोजेब भटमासको कोसा कुहिने रोग व्यवस्थापन साथै उत्पादन वृदिमा प्रभावकारी रहेको पाइयो ।

## INTRODUCTION

Soybean (*Glycine max* L. Merril) is an important summer legume of mid hill, grown as an intercrop with maize or in paddy bund that occupies about 80% in terms of total soybean area and production in Nepal (GLRP 2012). Soybean occupies an area of about 24934 ha with total production of 29221 mt with an average productivity of 1172 kg/ha (MoAD 2013), which is far less than global average productivity of 1.712 tons/ha (Masuda and Goldsmith 2008). For poor productivity prevailing diseases are one of the main reasons in Nepal. It is known that more than hundred pathogens affect soybean crop, some of them causing 10-12% yield losses (Mittal et al 1993). Among those Anthracnose disease caused by *Colletotrichum truncatum* in soybean is one of the most important seed-borne fungal pathogens appears late in season on stem, pods and seeds (GLRP 2011). The disease causes reduction of seed germination, seed quality and severe yield losses may occur in warm and humid regions of tropics and subtropics (Sinclair 1989) where soybean production is presently expanding.

In India, anthracnose is considered the most serious soybean disease (Khare and Chacko 1983). In the southern United States, this disease caused estimated yield losses ranging from 0.1 to 7.0% in the growing seasons (Koldenhoven et al 1983, Mulrooney 1985, 1986, 1988). Estimates of maximal seed yield reduction to anthracnose ranged from 16 to 26% in susceptible cultivars in Alabama (Backman et al 1982). Yield losses of 30 to 50% were reported in Thailand and 100% in India (Sinclair 1989).

Although several soybean lines were rated as resistant to the pathogen, no germplasm accessions tested so far have been shown to be immune (Manandhar et al 1988). *C. truncatum* is seed-borne and seed-transmitted disease which might cause systemic infection (Neergard 1979). The pathogen over-winter in crop debris (Athow 1973, Sinclair 1989) and several weed species may also serve as sources of inoculums (Hartman et al 1986, McLean and Roy 1988). Management of plant disease successfully achieved through application of chemical fungicides. Although, pod blight in soybean results in severe yield losses, however, very few works with respect to disease management have been carried out to cope of this disease in Nepal. So, the present experiment was undertaken to evaluate efficiency of available fungicides in market against soybean anthracnose disease under field conditions.

## MATERIALS AND METHODS

The experiments were conducted during summer of 2012-2014 at Grain Legumes Research Program, Rampur, Chitwan by laying out in a well managed piece of land using Randomized Complete Block Design having five treatments with different fungicides against one control with four replicates of each. During soybean season, a susceptible variety Ransom was planted on second week of July in a unit plot size of 3m x 2m and 50 cm x 10 cm spacing. The fungicides were Dithane M-45 (Mancozeb 75% WP) @ 2.5 g/l, Krilaxyl (Metalaxyl

8%+ Mancozeb 64% WP) @ 2 g/l, Bavistin (Carbendazim 50% WP) @ 2g/l, Blitox-50 (Copper oxy chloride 50% WP) @ 3 g/l and SAAF (Carbendazim 12% + Mancozeb 63%) @ 2.5 g/l.

After completion of the sowing, the plots were kept under constant supervision from sowing to harvest. Agronomic practices were followed as recommended. First spray was given just after the appearance of disease symptom in the field. Three sprays were given at an interval of 15 days. Data were recorded before every spray using 1-9 scoring scale on 5 randomly tagged plants/plot. The control plots received no fungicides. Percent Disease Index (PDI) was computed according to the formula (Wheeler 1969) and calculation was based on the final data recorded at 15 days after the last spray.

Percent Disease Control (PDC) was calculated on the basis of the formula developed by Shivankar and Wangikar (1993). Early Plant Stand (EPS) and Final Plant Stand (FPS) were recorded. At harvest, data on total number of pod per plant, number of infected and healthy pods, 100 seed weight and seed yield were recorded with yield data presented in terms of hectare. Yield increase over the control was calculated. All data were analyzed statistically using MSTAT-C computer package program. Treatment means were compared using Least Significance Difference (LSD) and Duncan's Multiple Range Test (DMRT) at 5% levels of significance. The correlation among percent yield increased over control and percent disease control was calculated.

## RESULTS

### Efficacy of Fungicides In-vivo

The lower PDI with a value of 42.53% was recorded in plot treated with SAAF. The second most effective 47.25% of PDI was occurred in Mancozeb treated plants, while and highest value of 73.57% was in control. Final plant stand was also noticed higher in plot treated with SAAF in 47.25% while 45.75% in plots treated with Mancozeb. In SAAF treated plot the Percentage Disease Control (PDC) and Percent Yield Increase (PYI) were 42.19% and 108.69%, respectively followed by Mancozeb compared to control plot. The mean Pod Infection Percentage was noticed lower with a value of 22.31% in plot treated with SAAF followed by Mancozeb with 23.04% of value (Table 1).

**Table1.** Effect of fungicides on pod blight disease severity and yield performance of soybean at Rampur, Chitwan, Nepal in 2012/13

| Treatments | Early Stand | PDI (%) | Pod/ plant | Final Stand | Yield Kg/ha | HSWT (g) | PI (%) | PDC (%) | PYI (%) |
|---|---|---|---|---|---|---|---|---|---|
| SAAF (2.5g/l of water) | 50.25 | 42.53$^{c}$ | 51.50$^{a}$ | 47.25$^{a}$ | 1500.00$^{a}$ | 15.45$^{a}$ | 22.31$^{d}$ | 42.19 | 108.69 |
| Mancozeb (2.5g/l of water) | 46.75 | 47.25$^{c}$ | 48.50$^{ab}$ | 45.75 $^{ab}$ | 1302.08$^{b}$ | 14.62$^{b}$ | 23.04$^{d}$ | 35.77 | 81.16 |
| Krilaxyl (2g/l of water) | 51.50 | 59.40$^{b}$ | 45.25$^{b}$ | 41.75$^{ab}$ | 1000.00$^{c}$ | 13.44$^{d}$ | 33.59$^{bc}$ | 19.26 | 39.13 |
| Blitox-50 (3g/l of water) | 46.50 | 48.60$^{c}$ | 47.50$^{b}$ | 42.75$^{ab}$ | 1062.50$^{c}$ | 13.97c | 32.82$^{c}$ | 33.94 | 47.83 |
| Bavistin (2g/l of water) | 42.75 | 68.62$^{a}$ | 32.00$^{c}$ | 40.00$^{bc}$ | 864.58$^{d}$ | 13.27$^{d}$ | 38.76$^{b}$ | 6.73 | 20.29 |
| Control | 51.25 | 73.57$^{a}$ | 25.25$^{d}$ | 34.00$^{c}$ | 718.75$^{e}$ | 12.41$^{e}$ | 73.92$^{a}$ | | |
| CV,% | 12.40 | 8.73 | 5.71 | 10.37 | 7.81 | 1.17 | 9.93 | | |
| LSD (0.05) | | 7.46 | 3.58 | 6.55 | 126.42 | 0.24 | 5.60 | | |

Means in column with same superscript is not significantly (P<0.05) different by DMRT (Duncans Multiple Range Test). PDI- Percent Disease Index, FPS – Final Plant Stand/Plot, Yield (Kg/ha) - Grain yield in Kilogram per hectare, HSWT (gm) - Hundred Seed Weight in gram, PI% - Mean pod infection percentage, PDC - Percent Disease Control, YI - Yield Increase, g/lt – Gram per litre

### Relationship between Percent Disease Control (PDC) and Percent Yield Increase (PYI)

In year 2013, the trends of disease control and yield observations were almost similar comparing to previous year. The PDI, number of pods per plant, final stand, yield, 100 seed weight and mean pod infection were significantly ($P<0.05$) varied with the treatments. The lower (46.25%) PDI was recorded on plots sprayed with SAAF, the values increased when treated with Mancozeb having a value of 53.42% and then others. The maximum Mean Pod Infection percentage of 75.94% was recorded in control plot, while minimum in SAAF treated plot having a value of 29.67% and then in plot treated with Mancozeb (with a value of 33.15%), respectively. The yield of 2431.25 kg/ha and seed weight of 13.52 g/100 were also significantly ($P<0.05$) higher in plots treated with SAAF and then by Mancozeb sprayed plots. The lower yield was recorded from control plot (743.75 kg/ha) (Table 2).

A linear negative correlation between yield and PDI was observed representing the best fit having $R^2$=77% (Figure 2). Obviously the yield decreased with the increase in Percent Disease Index (PDI). The linear correlation between PDC and PYI showed positive correlation ($R^2 = 0.796$) (Figure 1) showing that control in disease enhance the yield.

**Table 2.** Influence of fungicides on pod blight disease severity and yield performance of soybean at Rampur, Chitwan during 2013/14

| Treatments | Early Stand | PDI (%) | Pod/ plant | Final stand | Yield kg/ha | HSWT (g) | PI % | PDC (%) | PYI (%) |
|---|---|---|---|---|---|---|---|---|---|
| SAAF (2.5 g/l of water) | 147.25 | 46.25$^{d}$ | 48.90$^{a}$ | 149.00$^{a}$ | 2431.25$^{a}$ | 13.52$^{a}$ | 29.67$^{b}$ | 37.13 | 226.89 |
| Mancozeb (2.5 g/l of water) | 153.75 | 53.42$^{c}$ | 46.07$^{ab}$ | 139.50 $^{b}$ | 1522.06$^{b}$ | 12.60$^{b}$ | 33.15$^{b}$ | 27.38 | 104.64 |
| Krilaxyl (2 g/l of water) | 168.50 | 54.22$^{c}$ | 43.42$^{bc}$ | 131.75$^{c}$ | 1430.31$^{d}$ | 11.65$^{c}$ | 35.71$^{b}$ | 26.30 | 92.31 |
| Blitox-50 (3 g/l of water) | 169.75 | 53.58$^{c}$ | 45.25$^{abc}$ | 134.00$^{bc}$ | 1477.19$^{c}$ | 12.50$^{b}$ | 34.04$^{b}$ | 27.17 | 98.61 |
| Bavistin (2 g/l of water) | 176.25 | 65.07$^{b}$ | 41.97$^{c}$ | 128.50$^{c}$ | 1373.55$^{e}$ | 11.49$^{c}$ | 36.21$^{b}$ | 11.55 | 84.67 |
| Control (Untreated check) | 158.00 | 73.57$^{a}$ | 34.00$^{d}$ | 107.75$^{d}$ | 743.75$^{f}$ | 9.38$^{d}$ | 75.94$^{a}$ | | |
| CV, % | 9.04 | 4.01 | 5.52 | 2.93 | 1.59 | 1.91 | 18.34 | | |
| LSD (0.05) | | 3.48 | 3.60 | 5.82 | 35.86 | 0.34 | 11.28 | | |

Means in column with same superscript is not significantly ($P<0.05$) different by DMRT (Duncans Multiple Range Test) PDI- Percent Disease Index, FPS – Final Plant Stand/plot, Yield (kg/ha)- Grain yield in Kilogram per hectare, HSWT (g)- Hundred Seed Weight in gram, PI% - Mean pod infection percentage, PDC- Percent Disease Control, YI- Yield Increase.

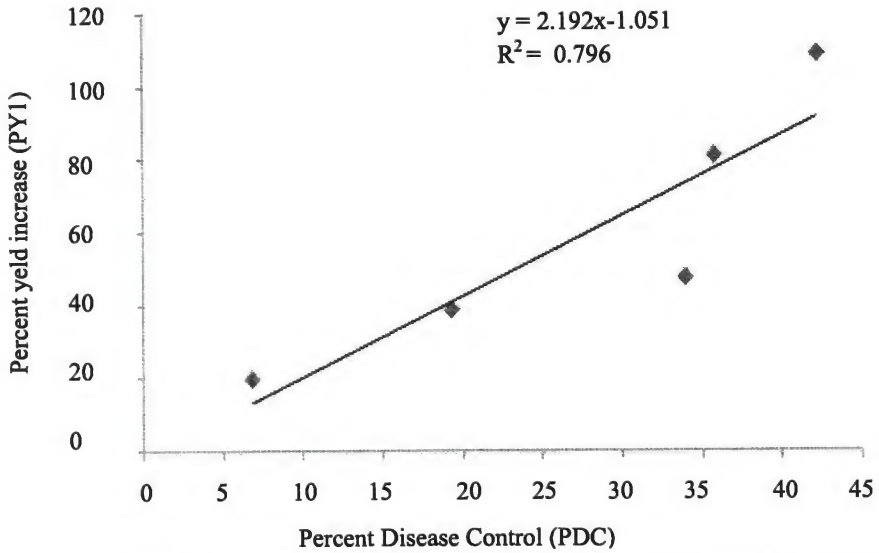


**Figure 1.** Relationship between PDC and PYI of fungicides used in soybean anthracnose.

## DISCUSSION

Chemical treatment is one of the most recommended methods used for plant disease management. The present experiments with various available chemicals on Soybean crop for two consecutive years clearly showed low Percent Disease Index (PDI) and higher yield from the plot treated with SAAF (Carbendazim 12% + Mancozeb 63%) @ 2.5 g per litre of water comparing to control. This findings is in close agreement with Chakraborty and Shyam (1988) and (Ghawde et al 1996), who showed that fungicide Carbendazim was inhibitory to the *Colletotrichum lindemuthianum* causing anthracnose to French bean, *C. truncatum* The fungi *Colletotrichum graminicola* and *C. capsici* cause blight in bitter gourd (Singh and Dwivedi 2002, Dubey and Ekka 2003) while, *C. gloeosporioides* causes anthracnose in mango (Kumar et al 2003). Application of fungicides to control anthracnose disease of soybean reduces the yield losses (Backman et al 1982). Efficacy of fungicides in controlling anthracnose disease and increasing the yields were reported earlier (Khare et al 1972, Chaudhary 1977, Palarpawar and Ghurade 1989, Kumar and Mukhopadhyay 1990, Bhardwaj and Thakur, 1991, Shukla and Singh 1993, Dubey and Ekka 2003, Ekbote 2005).

It is clear that soybean sprayed with fungicides had lesser disease severity with increase in yield than untreated ones. The SAAF and Mancozeb @ 2.5 g/l of water were found best among the sprayed fungicides. The lesser percent disease index, lower mean pod infection percentage along with the higher yield and 100 seed weight was found from the plot applied with SAAF followed by Mancozeb. It is recommended that soybean field should be sprayed by SAAF @2.5 g/l of water twice at vegetative and pod formation stage against anthracnose/pod blight disease for maximum yield with the reduction of disease severity.

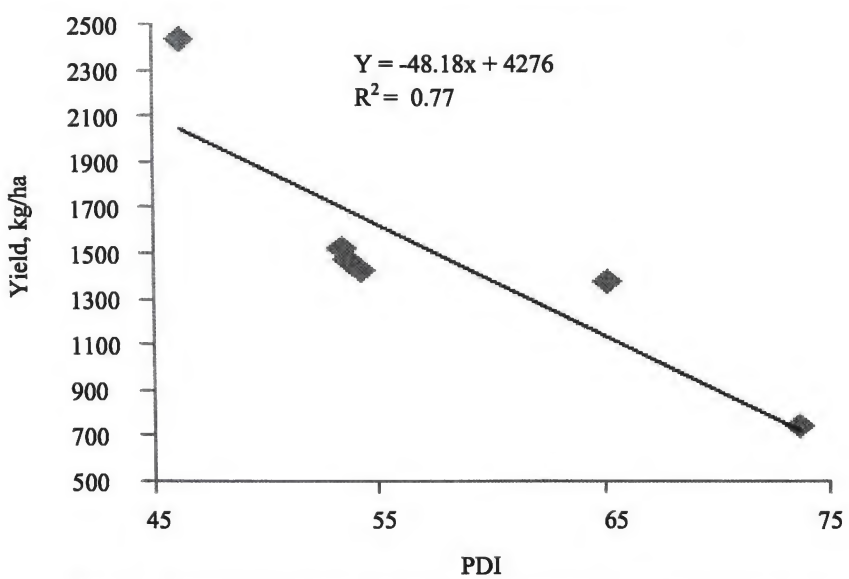


**Figure 2.** Relationship between Percent Disease Increase (PDI) and yield of soybean.

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# Yield Interactions of Wheat Genotypes to Dates of Seeding in Eastern Mid Hills of Nepal

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## ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the major cereal crops and staple food sources in Nepal. Wheat varieties being popular in mid hill regions are still in the early stages of adoption. Identification of appropriate date of seeding plays important role in enhancing the adoption rate ensuring the sustainable production. Therefore, three dates viz 15th November, 1st and 15th December for seeding and twenty eight wheat genotypes were evaluated in a split plot design with two replications for two consecutive seasons in 2011/12 and 2012/13 at an altitude of 2200 masl of eastern Nepal. The results showed genetic differences and interaction effect of genotypes with the dates of sowing on grain yield, panicle length and effective tillers per square meter. The wheat sown on 1st December showed the highest yield as compared to other sown dates. Similarly, WK1907, WK1911, WK1803, WK1915, WK1909, WK1714 and WK1803 produced highest yield among the tested genotypes with retaining maximum number of effective tillers and posed suitable maturity across all sowing date.

**Key words:** Genotypes, planting date, wheat, yield

## सारांश

गहुँ नेपालको एक प्रमुख खाद्यान्न वाली हो । गहुँका नयाँ प्रचलित जातहरु कमै मात्रामा किसानको खेत वारीमा पुगेको पाईन्छ । सामान्यतया, गहुँ वालीको उत्पादकत्व स्थान र जातिय छनौट अनुसार पनि भरपर्ने हुन्छ । तसर्थ, उचित समय र स्थान अनुसारको उपयुक्त जातिय पहिचानको संयोजन वाट गहुँको वढी उत्पादन लिन सक्ने हुन सक्दछ । अझ नेपालका मध्य पहाड जस्ता भौगोलिक अवस्था रहेका स्थानहरुमा उचित समयको पहिचानका साथै गहुँको उपयुक्त जातको समन्वय उत्कृष्ट उत्पादन कालागी एक महत्वपुर्ण जानकारी हुनाका साथै सो वाट उत्पादनको दिगोपनाको सुनिश्चतता प्रदान गर्दछ । यी सवै जानकारी हासिल गर्ने उद्देश्य अनुरुप १५ नोभेम्वर, १ डिसेम्वर र १५ डिसेम्वरको फरक मितिहरुमा २८ गहुँका जातहरु लाई स्पलिट प्लट डिजाईनमा तिन रेप्लिकेशन विधिद्वारा सन् २०१२ र २०१३ मा कृषि अनुसन्धान केन्द्र पाखीवासको २२०० मिटर समुद्र सतहमाथि अवस्थित प्लटहरुमा परीक्षण गरिएको थियो । दुई वर्ष सम्म संचालन भएको परीक्षणले गहुँको जातिय भिन्नता र विभिन्न मितिमा छरिएको प्रभाव वालाको लम्वाई, नलहरुको संख्या र उत्पादनमा आधारित प्रक्षेपण गरि मूल्यांकन गर्दा १ डिसेम्वरमा छरिएको गहुँका २८ जातहरु मध्येमा सवैभन्दा वढी उत्पादकत्व भएकाहरुमा WK1907, WK1911, WK1803, WK1915, WK1909, WK1714 र WK1803 रहेको निक्यौल गरियो ।

## INTRODUCTION

Wheat is third most important major staple food crop in Nepal. The wheat crop supplements 20% of food calories contributing food to 36% of the global population. Productions of wheat crop in the country have reached approximately 1883147 mt from 754474 ha of cultivated land with 2.496 t/ha yield (ABPSD 2013/2014). The national statistics shows that the average productivity of wheat in eastern hills was about 2.44 t/ha which is lesser as compared to other hilly regions and national yield (MoAC 2013). In general, wheat grain yield highly depends on water availability, high or low temperatures during the grain filling at maturity and other factors (Gomez-Macpherson and Richards 1995, Andarzian et al 2014) Thus, the identification of sowing date in relation to wheat genotype is an important management option to optimize grain yield potential (Ahmad and Fayyaz-ul-Hassan 2015).

Moreover, cold temperature related sterility is the major reason behind the yield loss where proper adjustment of planting date failed (Sthapit 1988). According to NWRP (2013), a delay of fifteen days from 21st November might reduce the wheat production by 20 to 30%, implying that, identification of the appropriate sowing date to plant wheat genotypes in specific agro-ecological conditions has high implication associated with sharp vertical gradients under mid hill condition of the country. Therefore, the present experiments, as we have aimed possess high importance to find out appropriate sowing dates in association with suitable genotypes in the mid-hill environment.

## MATERIALS AND METHODS

Field experiments were laid out during the wheat production seasons in 2011 to 2013 at Agricultural Research Station, Pakhribas, Dhankuta, Nepal located at 2200 masl using split plot design with two replications. Three different seeding dates (15th November, 1st December and 15th December) were used as main-plot and 28 genotypes were placed as sub plot factor. The genotypes comprised of wheat advanced lines from Khumal series, facultative wheat genotypes namely, 18th FAWONN 222 and 18th FAWONN 246, whereas WK1204 was used as popular check variety common to the mid-hill condition of Nepal (NWRP 2013). All the genotypes were obtained from Agriculture Botany Division.

The fertilizers were applied at the rate of 100: 60: 40 kg N: $P_2O_5$:$K_2O$ (Nitrogen: Phosphorus: Potassium) $ha^{-1}$ and the seed rate was 120 kg $ha^{-1}$. All other agronomic practices, e.g. method of planting, seeding rate, planting depth, weeding and harvesting were kept constant for all treatments. The experimental plot consisted of 6 rows of 2 meter long where continuous seeding was done manually. Ten plants from each plot were randomly selected to record the data for plant height and spike length. Similarly, yield was computed by weighing the weight of seeds per spike from four rows of each plot. The major measured traits were number of effective tillers per square meter, maturity days and yield. The number of effective tillers per square meter was calculated by counting tillers during the harvest from one square meter while maturity days were calculated by counting the days between dates of sowing and date to 95% physiological maturity. The plants were harvested and measured separately from different sown date and traits; and computed using M-STAT Software. From the used parameters, mean, coefficient of variation (CV), least significant difference of mean and probability value were calculated.

## RESULTS

The levels of significance for different factors and their interactions for various measured traits were presented in Table 2. The analysis of variance showed that the mean squares for genotypes in all dates of sowing were significantly ($P<0.05$) different for yield (t/ha), number of tillers per square meter and maturity days indicating the existence of genetic variability (Table 2).

Moreover, number of tillers per square meter and yield (t/ha) were significantly ($P<0.05$) different at 1% level of significance among genotypes over the years. The general trend showed that 10 to 12 average day's diminution for number of days to 95% maturity with every week delayed in planting time (Table 2).

The wheat sown on 1st December yielded 308 tillers per square meter which was the highest among all, followed by sown on 15th November with 295 tillers per square meter. The wheat seeded on 15th December produced the lowest 274 tillers and minimum yield of grain 5.54 t/ha (Table 2), while seeded on 1st December yielded the highest 6.82 t/ha grain. Similarly, crop sown on the first date (15th November) took the highest number of days for maturation, comparing to the wheat sown on other second and third dates.

In comparison among the genotypes, WK1914 produced 394 tillers per square meter followed by WK1902 with 388 tillers per square meter. In addition to this, genotypes WK1905, WK1915, 18th FAWWON222, WK1713, WK1906, WK1901 and WK1907 produced more number of effective tillers per square meter with additional advantage of medium maturity days (Table 2). Among the genotypes, WK1723 was earliest in maturity (161 days) (Table 2). Genotypes WK1714, WK 1902, WK 1803 and WK 1723 produced higher grain yield among all sowing dates with 8.07, 7.66, 7.42 and 7.36 t/ $ha^{-1}$, respectively. Besides them, genotypes WK1907, WK1911, WK1915, WK1909 and WK1712 gave stable yield in all seeding dates.

## DISCUSSION

The result showed that the wheat genotypes sown on 1st December had highest yield with highest number of mean tillers per square meter than wheat sown in other dates, in general (Table 2). Our findings showed that wheat sowing can be recommended on around 1st December in high hill localities similar to that of the present experimental site. Similar to the present finding Subedi et al (1985) showed that wheat seeded on 6th December gave maximum yield in mid hill conditions of Nepal. The cumulatively results might imply with increasing altitude probably the wheat should be sown little earlier than approximately in late November, The early (15th November) and delayed (15th December) seeded wheat in present experiments could not exceed the yield of the 1st December. This could be due to yield loss associated with delayed or early sowing inappropriate for the specific mid hill location environmental interaction with the genotypes used. Since the yield is associated with the number of tillers, it has been found that number of tillers per unit area could be affected substantially in early or delayed seeding (Sharma and Garg 2002).

In general, similar results has been obtained elsewhere in late planted wheat because of the problem of field drought that hamper the production of tillers; and thus to the yield (Mohammad et al 2003). The delayed sown wheat produced low number of effective tillers per square meter probably due to drought and rise in temperature, as the plant faces increasingly warm temperature especially towards the maturation period, when plant start to enrich the grains. In present experiment the low number of tillers and yield in delayed sown wheat might be due to warming air temperature at the end of February, when day length inclined to be lengthen supporting rise in air temperature.

The genotypes WK1905, WK1914, WK1902, WK1915, 18TH FAWWON222, WK1713 WK1906, WK1901, WK1714, WK1803 and WK1907 had produced more number of effective tillers $m^{-2}$ (Table 2). The tiller numbers contributes in one or another way to grain yield (Bassu et al 2010). The higher number of tiller bearing trait could be one of the important characteristics for wheat improvement (Subedi et al 1985), because higher number of tillers favored more adaptation and production comparing to low tiller bearing genotypes. Therefore, tillers per square meter would be the most appropriate traits to be utilized for the wheat improvement.

Among genotypes, WK1714 and WK1803 matured in 145 and 162 days, with mean yield of 8.07 (ton/ha) and 7.42 (ton/ha), respectively (Table 2). The wheat sown on 15th November took higher number of days to mature than comparing to seeded in other dates. This is mainly because of cool temperatures persisted, which resulted in slow seedling emergence and slow subsequent development and fruiting. Similar observation has been mentioned by Subedi et al (1985) that low temperature resulted in slow vegetative and reproductive growth in plants. Since the air temperature is closely associated with the altitude, facing of crop fields to various directions, therefore care should be taken to imply the results of present experiments to be generalized elsewhere. More studies on wheat genotypes yield attribute at various altitudinal gradients in mid hill and southern terai might be of interest for rapid adoption of the wheat farming in applicable localities.

It has been calculated that mean number of days for maturation might be reduce by 12 days comparing to those sown on 15th November and 1st December. An enforced maturity has been noticed to the wheat sown on 15th December (Table 2). Similar observation has also been reported by Sandhu (1993), where duration of reproductive phase such as flowering and maturity were decreased with delaying in

seeding dates. The low yield in wheat sown on 15th November justified that specific timing would be slightly earlier for genotypes used for the specific mid hill localities because of persisting low temperature faced by wheat genotypes around December, January and February which do not stimulate positive attributes for yield performance in the specified locality.

The wheat sown on 1st December were matured in moderate duration comparing to delayed or forced maturity caused either by early or late sowing. This timing of seeding probably perfectly coincided with the yield attributes of genotypes used in specified mid hill localities. Moreover, this timing also perfectly coincided and fit to rice-wheat cropping system in mid hills of Nepal. Therefore, this timing should be recommended for the best yield performance of used genotypes (Table 2) in specific mid hill localities.

Genotypes WK1907, WK1911, WK1915, WK1909, WK1714 and WK1803 produced the highest yield along with at par yield to check variety, WK1204. maturing in medium duration and yielding highest tillers across the planting dates. Therefore, it is likely, these genotypes could be promoted as varieties under eastern mid hill condition of Nepal. Besides that, WK1907, WK1911, WK1915, WK1909, WK1714 and WK1803 were more stable genotypes, which could be utilized under advanced yield and subsequent promotional trials. In addition, based on present findings it is recommendable that the wheat genotypes preferably should be seeded in the first week of December for increased grain production in the eastern hills environments of Nepal.

**Table 2.** Interaction of wheat genotypes with different dates of sowing and their combined mean for grain yield (t/ha), number of tillers per square meter and maturity days during 2011/2012 and 2012/2013

| Genotypes | Yield (t/ha) | | | | Tillers/square meter | | | | Maturity days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 Nov | 1 Dec | 15 Dec | Mean | 15 Nov | 1 Dec | 15 Dec | Mean | 15 Nov | 1 Dec | 15 Dec | Mean |
| F-7@-45 | 6.97 | 8.22 | 6.02 | 7.07 | 317 | 255 | 268 | 280 | 176 | 166 | 152 | 165 |
| WK1712 | 7.25 | 7.11 | 5.13 | 6.50 | 248 | 254 | 235 | 246 | 177 | 165 | 153 | 165 |
| WK1713 | 7.68 | 7.60 | 5.30 | 6.86 | 316 | 335 | 294 | 315 | 186 | 166 | 149 | 167 |
| WK1910 | 7.10 | 8.43 | 5.63 | 7.05 | 239 | 246 | 206 | 230 | 182 | 166 | 169 | 172 |
| WK1906 | 3.07 | 4.56 | 2.30 | 3.31 | 299 | 343 | 291 | 311 | 194 | 185 | 168 | 182 |
| 18th FAWWON 222 | 5.69 | 6.10 | 5.27 | 5.69 | 414 | 379 | 300 | 364 | 192 | 180 | 169 | 180 |
| WK1907 | 4.77 | 5.88 | 5.82 | 5.49 | 283 | 342 | 240 | 288 | 199 | 183 | 168 | 183 |
| WK1905 | 5.74 | 5.10 | 5.08 | 5.31 | 424 | 446 | 411 | 427 | 186 | 181 | 159 | 175 |
| WK1976 | 6.54 | 5.75 | 5.03 | 5.77 | 272 | 254 | 245 | 257 | 188 | 172 | 149 | 170 |
| WK1723 | 7.78 | 7.96 | 6.35 | 7.36 | 222 | 288 | 249 | 253 | 168 | 164 | 152 | 161 |
| WK1901 | 7.66 | 6.39 | 5.96 | 6.67 | 342 | 290 | 282 | 305 | 184 | 168 | 153 | 168 |
| WK1909 | 6.39 | 6.78 | 6.15 | 6.44 | 256 | 259 | 207 | 241 | 182 | 167 | 156 | 168 |
| F(5)-1-I-3 | 6.25 | 6.08 | 6.10 | 6.14 | 204 | 256 | 262 | 241 | 182 | 167 | 144 | 164 |
| WK1714 | 10.1 | 8.13 | 5.98 | 8.07 | 307 | 217 | 205 | 243 | 173 | 169 | 169 | 170 |
| WK1904 | 7.48 | 6.75 | 4.92 | 6.38 | 300 | 298 | 303 | 300 | 189 | 180 | 145 | 171 |
| WK1792 | 5.16 | 4.92 | 4.97 | 5.02 | 282 | 312 | 275 | 290 | 172 | 163 | 153 | 163 |
| WK1804 | 7.64 | 7.62 | 6.70 | 7.32 | 289 | 324 | 234 | 282 | 175 | 172 | 155 | 167 |
| WK1204 | 8.59 | 8.63 | 6.41 | 7.88 | 268 | 270 | 247 | 262 | 182 | 167 | 156 | 168 |
| WK1030 | 4.67 | 5.99 | 5.98 | 5.55 | 276 | 291 | 278 | 282 | 186 | 172 | 158 | 172 |
| WK1902 | 8.84 | 7.96 | 6.17 | 7.66 | 390 | 438 | 336 | 388 | 175 | 174 | 156 | 168 |
| WK1914 | 5.16 | 6.30 | 5.35 | 5.60 | 396 | 438 | 348 | 394 | 196 | 179 | 168 | 181 |
| WK1710 | 6.20 | 7.01 | 5.71 | 6.31 | 252 | 276 | 236 | 255 | 182 | 163 | 150 | 165 |
| 18th FAWWON 246 | 6.25 | 7.16 | 4.49 | 5.97 | 282 | 268 | 228 | 259 | 185 | 169 | 160 | 171 |
| WK1915 | 7.26 | 7.10 | 4.81 | 6.39 | 336 | 402 | 370 | 369 | 182 | 167 | 163 | 171 |
| WK1901 | 7.06 | 6.65 | 6.81 | 6.84 | 185 | 260 | 249 | 231 | 182 | 167 | 154 | 168 |
| WK1803 | 8.28 | 7.96 | 6.01 | 7.42 | 271 | 312 | 363 | 315 | 182 | 168 | 153 | 168 |
| WK1481 | 5.71 | 6.17 | 5.93 | 5.94 | 258 | 256 | 250 | 255 | 180 | 167 | 153 | 167 |
| WK1713 | 7.65 | 6.60 | 4.77 | 6.34 | 331 | 303 | 259 | 298 | 182 | 167 | 154 | 168 |
| CV, % | | 17 | | 21.9 | | 15.2 | | 22.3 | | 2.1 | | 2.9 |
| LSD (0.05) | | 1.67 | | 3.2 | | 71.58 | | 126.5 | | 7.26 | | 9.913 |
| | | <0.001 | | 0.14 | | <0.001 | | 0.85 | | 0.031 | | 0.39 |

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*Research Note*

# Effects of Neem (*Azadirachta indic)* and Custard Apple (*Annona reticulata*) Diets on Sterility of House Rat (*Rattus rattus*)

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## ABSTRACT

Three different plant products diets – i) neem (*Azadirachta indic* A. Juss) oil mixed diet (neem oil mixed @ 80 ml/kg of normal diet), ii) neem seed powder mixed diet (neem seed powder mixed @ 80 g/kg of normal diet) and iii) custard apple (*Annona reticulata* L.) seed powder mixed diet (custard apple seed powder mixed @ 80 g/kg of normal diet) were separately fed to mature rats (*Rattus rattus*) with single dose feeding of 80 g per pair in a day on 13th week-age during the experimenting years, 2012/013 and 2013/014. In control group only normal diet without neem and custard apple constituents were fed. Sterility test of rat was conducted up to 38 and 28 weeks-age in first and second year, respectively. The test rats were fed normal diet during whole experimenting periods except the one day when they were fed only the neem or custard apple mixed diet on the age of 13th week. Efficacy of the mixed diets on rat-sterility was determined based on pregnancy and parturition by the rats. The two years' results confirmed that all the tested three mixed diets – neem oil mixed diet, neem seed powder mixed diet, and custard apple seed powder mixed diet were effective to stop pregnancy and parturition in rats during whole experimenting periods up to 38 and 28 weeks-age with single dose feeding of 80 g per pair (40 gm/rat) in a day on 13th week-age of the rats; whereas the pregnancy and parturition were observed in the rats that were fed only the normal diet. It is expected, neem and custard apple mixed diets can be utilized in reducing the economically important rodent populations in rice-wheat cropping system in future.

**Key words**: House rat, rat sterility, neem oil, neem seed powder, custard apple seed powder

## सारांश

मुसालाई नपुंसक तुल्याउन तीन थरिका वानस्पतिक आहारा (निमको तेल, निमको बिउको धुलो, सरिफाको बिउको धुलो) मिश्रृत दानाहरु छुट्टाछुट्टै ख्वाई मुसाको बाँझोपनमा पर्ने प्रभावकारीताको प्रयोग २०६९।०७० र २०७०।०७१ मा गरिएको थियो । उक्त परीक्षणमा दानाहरु तीन वटा र एउटा वानस्पतिक पदार्थ बिनाको दाना तयार गरी १३ हप्ता पुगेका पाको उमेरका मुसालाई १३ हप्ता उमेर पुगेको दिन ८० ग्राम प्रति जोडी (१ भाले र १ पोथी) का दरले घरमुसालाई प्रयोगशालामा ख्वाईएको थियो । अरु दिन परिक्षण अवधिभर मुसालाई वानस्पतिक पदार्थ मिश्रण नगरिएको दाना ख्वाईएको थियो । परीक्षण कार्य मुसाको उमेर आ. व. २०६९।०७० मा ३८ र २०७०।०७१ मा २८ हप्ता पुरदा सम्म संचालन गरिएको थियो । मुसाको बाँझोपनको जाँच मुसामा गर्भ रहने तथा सन्तान उत्पादन गर्ने क्षमताको आधारमा गरिएको थियो । दुई वर्षको परिक्षणबाट प्राप्त नतिजाको आधारमा प्रयोग गरिएका तीन वटै (निमको तेल मिश्रृत दाना (निमको तेल ८० मि.लि. प्रति किलोग्राम दाना), निमको बिउको धुलो मिश्रृत दाना (निमको बिउको धुलो ८० ग्राम प्रति किलोग्राम दाना) र सरिफाको बिउको धुलो मिश्रृत दाना (सरिफाको बिउको धुलो ८० ग्राम प्रति किलोग्राम दाना) ले ८० ग्राम प्रति जोडी तथा ४० ग्राम प्रति मुसाका दरले मुसालाई १३ हप्ता पुगेको दिन एकपटक मात्रै ख्वाउँदा परिक्षण अवधिभर मुसामा गर्भ र सन्तान उत्पादन रोक्न प्रभावकारी हुने पाईयो । जवकी वानस्पतिक पदार्थ मिश्रण नगरिएको दाना ख्वाईएका मुसामा नियमित रुपमा गर्भ तथा सन्तान उत्पादन भएको पाईयो । यो प्रविधि भविष्यमा खास गरी धान पछि गहुँ लगाउने खेती प्रणालीमा मुसा नियन्त्रण गर्न उपयोगी हुन सक्ने संम्भावना देखिन्छ ।

## INTRODUCTION

More than 6000 species of rodents are reported worldwide among which 600 species belonging to genus *Rattus* are called rats (Fall 1977). Thirty species of rodents (rat and mice) are reported in Nepal out of them five are the economically most important common field and domestic rats and mice to damage the agricultural produce in fields and households (Joshi et al, 1991). They are the: house rat (*Rattus rattus*), house mouse (*Mus musculus*), brown rat (*Rattus norvegicus*), lesser bandicoot rat (*Bandicota bengalensis*) and large bandicoot rat (*Bandicota indica*) (Joshi et al 1991). Study on reproductive biology (Shrestha 2001) showed that successful mating of house rat was better in spring (90%) and autumn (90%) as compared to summer (80%) and winter (70%). In those studies, gestation period of house rat was 20-23 days; and male and female were sexually matured on 11th and 13th week after birth, respectively.

Enormous losses is caused by rodents in various ways by - damaging the standing crops and stored products; picking the sown seeds in fields; carrying the food materials in their burrows; spoiling the food grains with their excreta, urine and hair; extensive burrowing the crop fields; damaging the cloths and weaker storage structures; transmitting the rodent born diseases to human and pet animals etc (Joshi et al 1991, Ghosh and Durbey 2003, Lakshminarayanan et al 2015). In India, postharvest loss due to rodents was estimated to be 25-30%. In a 100 x 100 $m^2$ godowns, the loss due to rodents could be 4,200 kg estimating that 6 rats can consume the food equal to one man (Ghosh and Durbey 2003).

Rodents mainly the *Rattus rattus*, *Bendicota bengalensis*, and *Mus musculus* are agriculturally more important in Nepal causing the agricultural losses up to 15-25% every year (Shrestha 2001). Shrestha (2001) showed that the movement of house rat in rice and wheat fields in periphery of houses was up to 100 and 150 m, respectively. Yield losses caused by rodents in rice and wheat fields were estimated to be 575 kg/ha and 1132 kg/ha, respectively (Shrestha 2001). The total grain loss in storage by rat was estimated to be 2996 mt per year in Kathmandu valley (Shrestha 2001).

Several methods are available to control rats in fields and households (Joshi et al 1991, Upadhyay et al 1993, ED 1995/96, Mahajan et al 2015). However, in present experiments to minimize the rodent populations in fields and households, we approached method to cause sterility in rats by using botanical-mixed diets. For the purpose series of preliminary works were done in the past (ED 2008, Shrestha 2010, ED 2012). Here, in this paper we elucidate efficacy of some botanical-mixed diets on sterility of house rat.

## MATERIALS AND METHODS

### Preparation of Test Rats

House rats (*Rattus rattus*) were used as test rats that were collected from the farmers' households. They were kept in cages singly for three weeks in laboratory condition and were reared by feeding the normal diet and water at Entomology Research Division. Khumaltar, Lalitpur, Nepal. After the female rats were recognized not to be pregnant each of them were reared together with a mature male in separate cage for 10 days. The pregnancy of the female rats was checked by pressing the thumb in abdominal cavity. The pregnant rats were reared singly in separate cages until parturition. The newly born young rats were reared together with their mother up to 28 days. The young rats were then separated from their mother and were reared in pair (1 male and 1 female) or in groups until they get matured on 90 days (13 weeks) to be used for sterility test.

### Preparation of Normal and Botanical Mixed Diets

Neem (*Azadirachta indica* A. Juss) seeds and Neem oil were collected in July while Custard apple (*Annona reticulata* L.) seeds were collected from August to September from local market. These seeds were separately shade dried and crushed into powder. Normal diet was prepared by mixing the various ingredients at the proportion of 400 g of chicken feed (No: 3), 200 g of whole wheat meal, 200 g of grinded gram, 100 g of broken pieces of groundnut, 60 g of skim milk, 20 g of sodium chloride and 20 ml of mustard oil. The normal diet was used for two purposes - one for maintenance & mass production of experimental rats, and another for control diet to be used as a treatment in sterility test of the rats. The two ingredients - groundnut pieces and mustard oil may not be required for preparing the maintenance and mass production diet.

The normal diet was cooked in boiling water and fed to rats daily for maintenance and mass production. Three different diets – i) neem seed powder mixed diet (neem seed powder mixed @ 80 g per kg of normal diet), ii) custard apple seed powder mixed diet (custard apple seed powder mixed @ 80 g per kg of normal diet), and iii) neem oil mixed diet (neem oil mixed @ 80 ml per kg of normal diet) were prepared for efficacy test on rat-sterility. The four diets (one control/normal and three botanical mixed diets) that were to be used as four treatments in sterility test were prepared in the form of pellets each of 10 g dry weight (11.5 g of wet weight) with the help of pellet making frames. The wet pallets of 11.5 g were oven dried at 60°C for 5 hours or until to dry.

### Test of Botanical-Mixed Diets on Sterility of House Rat

Each treatment diet was fed to four pairs (as four replications) of matured rats in a day on 13$^{th}$ week-age with single dose feeding of 80 g (8 pellets each of 10 g) per pair in laboratory/room condition during the experimenting years, 2012/013 (2069/2070) and 2013/014 (2070/2071). Each pair (1 male and 1 female) was kept in four separate cages as four replications – Cage 1, Cage 2, Cage 3 and Cage 4 (Tables 1, 2, 3, and 4). The experiment was conducted starting from 13$^{th}$ week-age and completing on 38$^{th}$ and 28$^{th}$ week-age of the rats, in 1$^{st}$ and 2$^{nd}$ year, respectively. The test rats were fed in normal diet during whole experimenting periods except the one day, when they were fed only the botanical-mixed diet on their 13$^{th}$ week-age. Rat-sterility was determined based on pregnancy and parturition by the rats. Pregnancy was checked by pressing the thumb in their abdominal cavity weekly. Body weight was also observed weekly. Parturition was observed based on gestation period regularly. The diet was supplied to rats at 3-4 PM daily.

## RESULTS

During the two years' experiments no pregnancy and parturition was observed in the rats that were fed the neem oil, neem seed and custard apple seed mixed diets. All the tested three mixed diets found effective to stop pregnancy and parturition in rats during whole experimenting periods up to 38 & 28 weeks-age of the rats with single dose feeding of 80 g per pair in a day on 13$^{th}$ week-age of the rats (Tables 1, 2, 3). Whereas, the pregnancy and parturition were observed in the rats that were fed only the normal diets (Table 4). The results of test diets on rat sterility were same in 1$^{st}$ and 2$^{nd}$ year, therefore representative data of only 1$^{st}$ year has been presented here. All the tested mixed diets were effective to stop birth in rats in both years.

## DISCUSSION

In preliminary studies (Shrestha 2010, ED 2008) all the three botanical-mixed diets - neem oil @ 20ml/kg of normal diet, neem seed powder @ 100 g/kg of normal diet, and custard apple seed powder @ 100 g/kg of normal diet were effective to stop birth in house rat during whole experimenting periods up to 40 weeks-age when each diet were fed @ 80 g per pair daily for two months in 2004/2005 and three months in 2005/2006. Later in a study (ED 2012) the same botanical-mixed diets that were used in the preliminary works were effective to stop birth in house rat and field rat (*Bendicota bengalensis*) during whole testing period up to 22 weeks-age when each botanical mixed diet were fed @ 80 g per pair daily for two weeks. Present study revealed that all the tested three botanical-mixed diets found effective to cause sterility in house rat during whole testing periods up to 38 and 28 weeks-age with single dose feeding @ 80 g per pair for one day on 13$^{th}$ week-age of the rats.

**Table 1.** Effect of neem seed powder mixed diet on pregnancy and parturition of house rat

| Age in weeks | Cage 1 | | | Cage 2 | | | Cage 3 | | | Cage 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Body wt (g) | | Preg-nancy | Body wt (g) | | Preg-nancy | Body wt (g) | | Preg-nancy | Body wt (g) | | Preg-nancy |
| | Male | Female | | Male | Female | | Male | Female | | Male | Female | |
| 13 | 100 | 85 | no | 90 | 100 | no | 140 | 90 | no | 130 | 120 | no |
| 14 | 105 | 90 | no | 100 | 110 | no | 140 | 90 | no | 130 | 130 | no |
| 15 | 105 | 100 | no | 100 | 115 | no | 140 | 90 | no | 140 | 130 | no |
| 16 | 110 | 100 | no | 105 | 115 | no | 150 | 100 | no | 130 | 125 | no |
| 17 | 105 | 100 | no | 110 | 120 | no | 155 | 110 | no | 130 | 130 | no |
| 18 | 130 | 120 | no | 150 | 130 | no | 160 | 130 | no | 145 | 150 | no |
| 21 | 150 | 120 | no | 150 | 150 | no | 170 | 150 | no | 150 | 150 | no |
| 24 | 170 | 150 | no | 160 | 160 | no | 180 | 160 | no | 160 | 160 | no |
| 25 | 170 | 160 | no | 165 | 160 | no | 180 | 165 | no | 165 | 160 | no |
| 27 | 180 | 165 | no | 175 | 165 | no | 190 | 170 | no | 180 | 165 | no |
| 30 | 185 | 170 | no | 200 | 195 | no | 200 | 175 | no | 200 | 190 | no |
| 32 | 180 | 165 | no | 210 | 190 | no | 200 | 185 | no | 190 | 180 | no |
| 35 | 190 | 170 | no | 220 | 200 | no | 220 | 200 | no | 210 | 195 | no |
| 37 | 190 | 180 | no | 220 | 200 | no | 210 | 200 | no | 210 | 210 | no |
| 38 | 210 | 190 | no | 225 | 200 | no | 210 | 200 | no | 210 | 210 | no |

**Table 2.** Effect of custard apple seed powder mixed diet on pregnancy and parturition of house rat

| Age in weeks | Cage 1 | | | Cage 2 | | | Cage 3 | | | Cage 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BW (g) | | Preg-nancy | BW (g) | | Preg-Nancy | BW (g) | | Preg-nancy | BW(g) | | Preg-nancy |
| | Male | Female | | Male | Female | | Male | Female | | Male | Female | |
| 13 | 190 | 80 | no | 13 | 110 | No | 100 | 80 | no | 120 | 90 | no |
| 14 | 100 | 85 | no | 14 | 110 | No | 100 | 90 | no | 140 | 80 | no |
| 15 | 110 | 110 | no | 15 | 120 | No | 100 | 110 | no | 140 | 100 | no |
| 16 | 120 | 100 | no | 16 | 125 | No | 120 | 100 | no | 135 | 95 | no |
| 17 | 125 | 95 | no | 17 | 135 | No | 140 | 95 | no | 135 | 95 | no |
| 18 | 140 | 120 | no | 18 | 140 | No | 150 | 120 | no | 160 | 110 | no |
| 21 | 150 | 140 | no | 21 | 160 | No | 170 | 140 | no | 170 | 125 | no |
| 24 | 160 | 150 | no | 24 | 170 | No | 200 | 180 | no | 190 | 140 | no |
| 25 | 180 | 160 | no | 25 | 180 | No | 210 | 170 | no | 190 | 150 | no |
| 27 | 190 | 170 | no | 27 | 190 | No | 220 | 185 | no | 200 | 170 | no |
| 30 | 210 | 180 | no | 30 | 200 | No | 210 | 180 | no | 180 | 180 | no |
| 32 | 220 | 180 | no | 32 | 200 | No | 210 | 185 | no | 200 | 190 | no |
| 35 | 220 | 195 | no | 35 | 210 | No | 210 | 170 | no | 200 | 195 | no |
| 37 | 220 | 195 | no | 37 | 220 | No | 210 | 175 | no | 200 | 195 | no |
| 38 | 220 | 190 | no | 38 | 210 | No | 230 | 180 | no | 200 | 200 | no |

**Table 3.** Effect of neem oil mixed diet on pregnancy and parturition of house rat (2012/013)

| Age in weeks | Cage 1 | | | Cage 2 | | | Cage 3 | | | Cage 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BW(g) | | Preg-nancy | BW (g) | | Preg-Nancy | BW(g) | | Preg-nancy | BW (g) | | Preg-nancy |
| | Male | Female | | Male | Female | | Male | Female | | Male | Female | |
| 13 | 110 | 100 | no | 100 | 100 | No | 100 | 90 | no | 100 | 100 | no |
| 14 | 130 | 135 | no | 130 | 110 | No | 120 | 110 | no | 120 | 110 | no |
| 15 | 145 | 145 | no | 140 | 135 | No | 155 | 125 | no | 135 | 115 | no |
| 16 | 150 | 150 | no | 155 | 150 | No | 160 | 120 | no | 140 | 120 | no |
| 17 | 165 | 150 | no | 160 | 150 | No | 165 | 135 | no | 155 | 135 | no |
| 18 | 165 | 150 | no | 165 | 150 | No | 170 | 145 | no | 160 | 140 | no |
| 21 | 170 | 155 | no | 165 | 150 | No | 180 | 150 | no | 160 | 150 | no |
| 24 | 175 | 155 | no | 170 | 155 | No | 185 | 155 | no | 165 | 145 | no |
| 25 | 175 | 155 | no | 170 | 155 | No | 190 | 155 | no | 170 | 150 | no |
| 27 | 185 | 165 | no | 185 | 170 | No | 200 | 165 | no | 185 | 165 | no |
| 30 | 180 | 170 | no | 200 | 170 | No | 210 | 175 | no | 195 | 170 | no |
| 32 | 200 | 200 | no | 230 | 170 | No | 210 | 175 | no | 230 | 185 | no |
| 35 | 210 | 200 | no | 230 | 190 | No | 230 | 200 | no | 240 | 185 | no |
| 37 | 210 | 190 | no | 235 | 195 | No | 230 | 200 | no | 240 | 195 | no |
| 38 | 210 | 200 | no | 240 | 200 | No | 240 | 200 | no | 250 | 200 | no |

**Table 4.** Effect of normal diet on pregnancy and parturition of house rat

| Age in weeks | Cage 1 | | | Cage 2 | | | Cage 3 | | | Cage 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Body wt (g) | | Preg- | Body wt (g) | | Preg- | Body wt (g) | | Preg- | Body wt (g) | | Preg- |
| | Male | Female | nancy | Male | Female | nancy | Male | Female | nancy | Male | Female | nancy |
| 13 | 110 | 100 | no | 100 | 100 | no | 110 | 100 | no | 100 | 100 | no |
| 15 | 125 | 120 | no | 120 | 120 | yes | 125 | 120 | yes | 130 | 130 | yes |
| 17 | 155 | 145 | yes | 135 | 165 | yes | 155 | 135 | yes | 145 | 165 | yes |
| 18 | 160 | 160 | yes | 140 | 135 | par | 160 | 170 | yes | 155 | 120 | par |
| 19 | 160 | 185 | yes | 155 | 145 | no | 160 | 130 | par | 165 | 165 | yes |
| 23 | 165 | 135 | par | 160 | 150 | yes | 160 | 185 | yes | 170 | 130 | par |
| 25 | 165 | 170 | yes | 166 | 160 | yes | 175 | 140 | par | 190 | 180 | yes |
| 27 | 175 | 140 | par | 175 | 185 | yes | 185 | 170 | yes | 195 | 135 | par |
| 28 | 190 | 145 | no | 185 | 140 | par | 195 | 130 | par | 190 | 140 | no |
| 29 | 220 | 160 | yes | 195 | 150 | yes | 210 | 140 | no | 200 | 155 | no |
| 30 | 220 | 175 | yes | 200 | 160 | yes | 220 | 150 | yes | 210 | 165 | yes |
| 32 | 220 | 195 | yes | 210 | 185 | yes | 220 | 180 | yes | 220 | 180 | yes |
| 35 | 230 | 170 | par | 235 | 160 | par | 230 | 155 | par | 200 | 195 | yes |
| 37 | 245 | 175 | yes | 210 | 170 | no | 210 | 160 | no | 200 | 165 | par |
| 38 | 250 | 180 | yes | 225 | 175 | yes | 215 | 165 | yes | 210 | 175 | yes |

Rodent damage is very serious to agricultural produce both in fields and households (Upadhayay et al 2093, Lakshminarayanan et al 2015). Mainly the house rat and field rats are more destructive to standing crops/crop produce, stored grains and particularly to rice-wheat cropping system in Nepal. This study has achieved a possible alternative approach to reduce rodent populations and minimize the yield losses in crop fields and households particularly in rice-wheat cropping fields by causing sterility in them. This study confirmed that all the tested three botanical-mixed (7.5% of botanical powder or oil mixed) diets – neem oil mixed diet, neem seed powder mixed diet and custard apple seed powder mixed diet found effective to stop birth in house rat for whole testing periods up to 38 and 28 weeks-age by single dose feeding of 80 g per pair (40 g/rat) in a day on 13$^{th}$ week-age of the rats.

Several studies have shown effect of plant materials which have been successfully used to cause sterility in rats (Upadhayay et al 1993, Lakshminarayanan 2015, Mahajan et al 2015). In addition, this study has provided the clear protocols to prepare the diets for experimental and mass production of rats and preparing of test rats which will be applicable for the future studies. The present study has shown a preliminary experiment causing sterility in rats by single day feeding of the specific ration; however, more detailed physiological evidence based experiments should be performed in near future.

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*A Review Paper*

# Technological Advances in Huanglongbing (HLB) or Citrus Greening Disease Management

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## ABSTRACT

Huanglongbing (HLB), previously citrus greening disease, is the most destructive of citrus species causing major threat to the world citrus industry. The disease was reported from China in 1919 and now known to occur in more than 40 different countries of Asia, Africa, South and North America. Three species of gram negative bacterium namely *Candidatus Liberibacter asiaticus, Candidatus Liberibacter africanus* and *Candidatus Liberibacter americanus* are the casual organisms of HLB, respectively prevailing in the continent of Asia, Africa and South America. It is one of the most extensively researched subjects in citriculture world. HLB was detected in 2004 and 2005, respectively in San Paulo of Brazil and Florida of USA: the two leading citrus production hub of the world causing huge economic loss within 5 years of first detection. Since then research on HLB detection and management was further accelerated in American continents. This paper presents the scientific advancement made on detection, spread, economic losses caused by HLB in different parts of the world and controlling management strategies. Remarkable achievements have been made on HLB detection techniques including iodine test, qPCR and more recently in spectroscopy. While efforts are being made to develop resistance varieties using conventional and biotechnological tools management strategy which includes reduction of inoculums source, vector control and replant with disease-free planting materials still remains major option for HLB control. Citrus intercropping with guava have shown promising results for vector reduction.

**Key words:** Citrus, citrus greening, huanglongbing, liberibacter, management

## सारांश

ह्वाङ्लोङ्बिङ् (यच यल वि) रोग, सुन्तलाजात खेतीका लागि संसारभर मुख्य चुनौती हुदै आएको छ । यसको पुरानो नाम सिट्रस ग्रिनिङ रोग हो । सन् १९१९ मा सर्वप्रथम चीनबाट यस रोगको बारेमा विवरण प्रकाशित भएको थियो । हाल यो रोग एसिया, अफ्रिका, उत्तर तथा दक्षिण अमेरिकाका ४० भन्दा धेरै देशहरुमा फैलिसकेको छ । एसिया, अफ्रिका र दक्षिण अमेरिकामा क्रमसः क्यान्डिडाटस लिबेरिब्याक्टर एसियाटिकस, क्यान्डिडाटस लिबेरिब्याक्टर अफ्रिकानस र क्यान्डिडाटस लिबेरिब्याक्टर अमेरिकानस प्रजातिका ब्याक्टेरियाले यो रोग लगाउछ । ठूलो आर्थिक नोक्सानी गर्ने भएकाले सुन्तलाजात फलफुल बालीमा यस रोगको बारेमा अत्यन्तै धेरै अनुसन्धानहरु भएका छन् । यस बालीको व्यवसायिक उत्पादनका लागि संसारमा नै ठूला मानिएका ब्राजिलको सनपाउलो र संयुक्त राज्य अमेरिकाको फ्लोरिडा राज्यमा क्रमसः सन् २००४ र २००५ मा यो रोग देखिएको ५ वर्ष भित्रमा धेरै आर्थिक क्षति पुऱ्याए पछि यस रोगको अनुसन्धानले अमेरिकी महादेशमा अझै व्यापकता पायो । यस लेखमा यच यल वि रोगको पहिचान, फैलावट, यसले पुऱ्याउने आर्थिक क्षती र यो रोगको व्यवस्थापनका विषयमा गरिएका वैज्ञानिक प्रगतिका बारेमा चर्चा गरिएको छ । रोग पहिचानको लागि आयोडिन टेस्ट र पिसिआरको प्रयोग हुदै आएकोमा हालका वर्षहरुमा सजिलो र सस्तो स्पेक्ट्रोस्कोपी प्रविधिको प्रयोगमा पनि अनुसन्धान केन्द्रित भएका छन् । रोग नियन्त्रणका लागि परम्परागत र जैविक प्रविधिको प्रयोगबाट रोगअवरोधक जातको विकासका प्रयास भएपनि आशा अनुरुपको प्रतिफल प्राप्त नभएको परिप्रेक्षमा हालसम्म पनि रोगी बोटहरु हटाउने, रोगको कारक व्याक्टेरियाको संवाहक सिट्रस सिल्ला किरा नियन्त्रण गर्ने र निरोगी कलमी विरुवाको प्रयोग गरी पुनर्रोपण गर्नु नै यस रोगको व्यवस्थापनको विधि रहेको छ । यच एल बी रोगका सम्वाहक नियन्त्रणको लागि सुन्तला र अम्वा संगसंगै लगाउदा केहि आशाजनक नतिजा प्राप्त भएको छ ।

## INTRODUCTION

Globally, citrus fruit represents one of the most important commodities with respect to production and trade. In production volume, it occupies second position after banana and plantain (Statista 2012) and ranks top position in international trade in terms of value (UNCTAD 2011). In Nepal, citrus especially mandarin, sweet orange and lime, are among the commodities having high potential for commercial production. The mid-hill region of Nepal have favorable conditions for commercial production of citrus because of agro-climatic suitability, access to domestic and regional markets and past experiences of farmers on citrus farming. As a result of these reasons along with government priority for its commercialization the area under citrus cultivation and its total production have increased substantially over the years. In the year 2003/04, 148,010 mt of fresh citrus fruit was produced in Nepal from 24,799 ha area. The total area under citrus cultivation has increased to 36,975 ha and total production to 216,188 mt in 2012. However, productivity of citrus decreased to 9.14 mt/ha in 2012 compared to 10.62 mt/ha in 2003 ((MoAD 2013).

Among many biotic factors contributing to low productivity and threat to citrus industry huanglongbing or greening disease, hereafter HLB has been considered the number one in Nepal (Regmi and Yadav 2007, Roistacher 1996) and the world (Bové 2006, 2014). HLB was first reported from southern China in 1919 and is now known to occur in more than 40 different Asian, African, Oceanian, South and North American countries (Bove 2006).

In Nepal, the disease was first reported from Pokhara in 1968 (Thrower 1968) as the main cause of citrus decline in the country (Roistacher 1996, Regmi and Yadav 2007). The disease is caused by a phloem-restricted, non cultured, Gram-negative bacterium transmitted by citrus psyllid insect vector; non-curable once trees are affected. Since HLB is very destructive disease and slowly invading new citrus growing areas represent a major threat to the world citrus industry including Nepal.

Mandarin (Suntala), sweet orange (Junar) and acid lime (Kagati) are the major commercial citrus species grown in Nepal (MoAD 2013). Since all citrus species are infected with HLB in varying degree and the disease is already spread several parts of the country (Bove 2006) understanding the complex nature of the disease and its management approaches is very vital to save the citrus industry of the country. In this context recent updates on technological information on detection and control management of HLB would be worth to be elucidated. Therefore, this paper presents a review on history, geographical distribution, detection and management of HLB.

## CAUSAL ORGANISM

Citrus greening disease or HLB was first reported from China in 1919 by Reinking while evaluating diseases of economic plants in southern China and used English term "yellow shoot" of citrusin the report, however, for a long period it (at that time the name "HLB" was not used) was thought to be present in China (Bove 2006). At that time it was believed that the HLB was caused by abiotic factors like Zn deficiency/toxicity and poor drainage system (Oberholzer et al 1965). By 1967, it became established that greening was graft and insect transmissible with conclusion caused by virus (Bove 2006). In 1967, mycoplasm like organisms (MLOs) were believed to be associated with plant diseases mostly with "yellow" symptoms resembling with greening symptoms. On close examination, these organisms were seen to have bacterial cell wall in addition to cytoplasmic membrane, suggesting that they were gram negative true bacteria (Garnier and Bove 1977). Thus, it was concluded that the HLB agent was gram negative bacterium. Later on it was confirmed by Electron Microscopy that South African "greening", Indian "dieback", Taiwan "likubin" and Philippines "mottle leaf" all were caused by HLB bacterium (Bove 2006).

Using universal primers, the 16s ribosomal DNA of two strains of the bacterium obtained by Polimerage Chain Reaction and comparisons with gene sequences showed the HLB organism belonged to the subdivision of the class Protobacteria, but were distinct from other members. The bacterium from Asia named Candidatus *Liberibacter asiaticus* (Las), and the African Ca. *L. africanus* (Laf) (Garnier et al 2000). Ca. *L. americanus* (Lam) is the new species identified in 2005 causing HLB in Brazil (Teixeira et al 2005).

The three species of HLB causing bacteria differ in their pathogenicity due to combination of environmental conditions and insect vectors (Jagoueix et al 1996). The African strain of HLB is heat sensitive and does not cause symptoms at temperatures greater than 25–$30^0$ C. The Asian strain, primarily distributed in Asia, is heat tolerant and able to cause symptoms at temperatures greater than $30^o$ C. The American strain, which is reported from Brazil and Mexico, appears to have heat tolerance similar to that of the African strain (NAPPO 2012).

## HISTORY

### Origin of HLB

Although HLB was first reported from China where many citrus species were originated most scientists believe that citrus species are not the original host of the HLB organism (Beattie et al., 2005, Bove, 2006 and Graca, 2011). There are different opinions among researchers about the origin of HLB. One of the hypothesis is that Asian HLB originated in citrus in India from unidentified native rutaceae and may have moved to other Asian countries through planting materials and citrus psyllids including China and other south-east Asian countries (Graca 2011).

Beattie et al. (2005) have proposed another hypothesis that the disease may actually originated in Africa, possibly in an asymptomatic host such as *Verpris lanceolata;* then transmitted to citrus by insect in European settlement on the east coast of Africa and then taken to the Indian subcontinent in infected plants or budwood 300 to 500 years ago, and then into China later. It has been recently suggested that the liberibacters have Gondwanan origins (Beattie et al 2005, Bove 2006).

At present HLB has spread to most of the citrus growing countries of the word. Only the areas free from HLB as well as of the psyllid vectors of the disease are Mediterranean basin, most of Western Asia (Near and Middle East), Australia, and North- and South-Pacific islands are still free of HLB (Bove 2006). Detail updated information of the disease distribution can be obtained from the website: *http://www.cabi.org/isc/ datasheet/16567.*

### China

Reinking (1919) used English word "yellow shoot" of citrus, a disease thought to be of little importance in those days. Later surveys showed that by 1936 the disease had spread to become a serious problem. The most extensive work on HLB in southern China was conducted from 1941 to 1955 by Lin Kung Hsiang (Bove 2006). From the discussions with the farmers of the Chaozhou county in Guangdong province, it was learned that HLB actually had been there since the 1870s. Based on this Lin postulated that HLB originated from Guangdong Province, China. He conducted several exploration in China and disclosed that farmers of Chaozhou district had given the name "huang long bing" to the disease, where "huang" means yellow, 'long' meant to 'dragon' and "bing" stands for disease. Hence, the disease was called "yellow dragon disease". The most outstanding result obtained by Lin was the demonstration, by precise experimental work, that HLB is a graft-transmissible, infectious disease, and should neither be attributed to physiological disorders such as mineral deficiencies or water logging, nor to soil-borne diseases such as nematode infestation or *Fusarium* infection (Bove 2006). For these reasons, the International Organization of Citrus Virologists (IOCV) accepted the official name of the disease be huanglongbing (HLB) in 1995 at the $13^{th}$ conference of the Organization in Fuzhou (Fujian, China).

### Africa

A disease similar to HLB, was observed in South Africa in 1928 under the name "greening". However, the true nature of the disease was not immediately recognized, and in the first description of "greening" in 1937, the problem was still assumed to be mineral toxicity (Oberholzer et al 1965). Through a collaborative research by A.P.D. McClean (phytopathologist) and P.C.J. Oberholzer (horticulturist)

demonstrated that greening was transmissible by graft inoculation as well as by the African citrus psylla, *T. erytreae*. The infectious nature of "greening" was thus established. The name "greening" was adopted by the scientific literature until "huanglongbing" was given as the official name in 1995, probably because of the proximity of western scientists to South Africa (Gottwals et al 2007).

### Philippines

The disease became a serious problem in the late 1950s was described in 1921 with the name - "mottle leaf" and thought to be related to zinc deficiency, because the symptoms of "mottle leaf" were very similar to those of HLB in China and Taiwan, and "greening" in South Africa (Salibe and Cortez 1968). Being graft-transmissible, HLB has also insect vectors: the African citrus psyllid, *Trioza erytreae*, reported as the vector in South Africa in 1965 and the Asian citrus psyllid, *Diaphorina citri*, identified as the vector in Asia (India and Philippines) in 1967 (Bové 2006).

### Other South-east Asian Countries

In Indonesia, HLB became a major problem identified first time in the name of "Vein phloem degeneration" or "phloem necrosis" in 1940s (Tirtawidjaja et al 1965). HLB first appeared in Thailand in the 1960s and was so severe that the length of time between the onset of the disease and debilitation of the entire tree was only about two years (Schwarz et al 1973). HLB disease, locally called "Likubin" was first identified in Taiwan in about 1930, but not considered as serious problem until 1957 (Gottwald 2007). The HLB inoculums was assumed to be brought into Taiwan from southern China through some infected citrus scions or seedlings and thought to be associated with poor soil conditions such as deficiency in essential nutrients or poor drainage (Su 2008). Other south Asian countries where HLB has also been detected by DNA hybridization and/or PCR include Myanmar, Malaysia, Cambodia, Laos and Vietnam (Bove 2006)

### India

Citrus in India has been known to suffer seriously from certain disorders resulting in low production, twig dieback, slow death and even sudden wilting attributed to "dieback", a disease that was first observed in the 18$^{th}$ Century in central India (Bove 2006). Proof for the presence of HLB in India was eventually obtained at the virus Research Center at Poona by Capoor and co-workers, when they succeeded in transmitting the HLB pathogen by the Asian psylla, *D. citri* by demonstrating that trees with dieback symptoms invariably proved positive for HLB (Capoor et al 1967). Thereafter, it was reported from different citrus growing regions of India and was considered to be principal cause of citrus dieback disease (Das 2008). From several surveys conducted from 2007 to 2012 along with molecular test (real-time PCR) in 16 states of India confirmed its distribution in all studied states (except Arunachal Pradesh): Andhra Pradesh, Assam, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura and West Bengal (Das et al 2014)

### Pakistan

Several surveys conducted during 1991 showed that citrus greening disease (CGD) is one of the most important and severe disease in Punjab and North West Frontier Province having an incidence rate of 22% in Kinnow, 25–40% in sweet orange, 15% in grapefruit, 10% in sweet lime, and 2% in lemon (Batool et al 2007).

### Nepal

Citrus decline was reported for the first time in Pokhara valley by Thrower (1968). Based on visual observation, Knorr et al (1970) suspected that the decline was caused by greening disease entered with the planting materials introduced to Horticulture Research Station, Pokhara from Saharanpur, India. About 55% of citrus trees in Pokhara valley and 100% in Horticulture Research Station were symptomatic to HLB in 1980s (Regmi 1982). Later more surveys and studies were carried out in other parts of the country to explore the distribution of greening disease and its vector (Regmi and Lama 1988, Regmi 1994, Regmi et al 1996). More recent PCR test showed that HLB is widespread in many citrus pockets of Kaski, Syanja, Tanahu, Lamjung and Dhading districts (Bove 2006, Regmi and Yadav 2007, Regmi et al 2010).

### America

Brazil has been the first American countries to report HLB, in March 2004 San Paulo State eliminated about 6 million infected trees by 2008, (Lopes et al 2011). The major HLB agent was the new liberibacter species Ca. *L. americanus*, present in 92% of the trees (Bove 2006). From 2005 on, the rest of America and the Caribbean islands started to be invaded by Las and *D. citri*: first Florida in 2005, then Cuba in 2006, followed by Mexico, Belize, Central America in 2009, Argentina, California, Guadeloupe andTexas (Bove 2014).

## DETECTION OF HUANGLONGBING

### Visual Symptoms

Citrus trees can be suspected to be affected with HLB by visual symptoms appeared on leaves and fruits (Bove 2006). A tree infected with HLB in the field usually develops one or more yellow shoots with other parts of the tree healthy or symptomless. The affected leaves develop a pattern of yellow and green areas lacking clear limits between the colors, giving a "blotchy mottle" appearance. This is the most characteristics foliar symptom and the patterns are asymmetrical on the two halves of the leaf (Bove 2006). Leaves can also become thicker, with veins enlarged and corky in appearance.

In later stages, Zn deficiency-like symptoms can be seen followed by leaf drop and twig dieback (Gottwald et al 2007). Symptomatic fruit are small, lopsided and as they mature and ripen the stylar end remains green, hence named as "greening" (Bove 2006). When cut in half, small, dark aborted seed can be observed and vascular bundles in the fruit axis are discolored. Fruit, especially sweet oranges, can also have mottle appearance and if the peel is pressed with finger, a silvering of the depressed area will result (Bove 2006). Visual symptoms of HLB noticed on leaves and fruits are varied and can be resemble other disorders like micronutrient deficiencies, such as zinc, manganese and iron (Etxeberria 2007).

Johnson et al (2014) found that greening causes a loss of 30-50% of trees' fibrous roots before symptoms are visible above ground. HLB bacteria enter trees through leaves but the disease attacks roots long before the leaves show signs of damage. Once bacteria enters through psyllid sucks into leaves, the bacteria travel quickly to the roots, where they replicate, damage the root system and spread to the rest of the host tree's canopy (Johnson et al 2014).

## Molecular Diagnosis

For the diagnosis of HLB, two molecular techniques have been used: conventional Polymerase Chain Reaction (PCR) and real time PCR (RT-PCR), also known as quantitative PCR (qPCR) that are based on the use of PCR primers that amplify DNA sequences of the Liberibacters associated with HLB (Bove 2006). Conventional PCR methods use specific primers that amplify the sequences of the rDNA 16s genes and primers based on the proteinaceous genes (operon-B) (Jagoueix et al 1996, Tian et al 1996, Hocquellet et al 1999, Teixeira et al 2005). The low concentration and irregular distribution of the pathogen in host plants, along with the inhibitors of PCR present in citrus extracts made the detection of the pathogen difficult. Although conventional PCR and qPCR are accepted techniques for the confirmation of trees symptomatic for HLB in Brazil and the United States, qPCR is considered much more sensitive and robust than conventional PCR and the technique has been validated with DNA extracts from different species of citrus and different tissues from diverse geographic regions (Li et al 2006, 2007).

## Biological Indexing

Although qPCR is currently the method of choice for diagnosis of HLB (Li et al 2006), biological indexing techniques are also available (Taba et al 2006). Due to low rate of graft transmission of the bacterium associated with HLB, the success rate for biological indexing of HLB is variable. The appropriate indicator plants are sweet orange or *Orlando tangelo* for African HLB and sweet orange or Ponkan mandarin for Asian HLB (Roistacher 1996). It is known that presence of the citrus tristeza virus can interfere with HLB symptom expression; and if CTV is present, grapefruit may be used as an indicator (Roistacher 1991). The preferred inoculation technique is the side graft, with leaf grafts being the alternative. The seedling indicators are trained to single leaders and held at $20 - 25^0$ C for African HLB and $25 - 32^0$ C for Asian HLB. Symptom expression is the typical mottle and chlorosis. The shoots are distinctly smaller, more chlorotic, and with smaller leaves than the uninoculated controls. Symptoms should appear 8 to 12 weeks after inoculation. Further details can be obtained in Roistacher (1991, 1996).

## Iodine Test

Although the PCR test for HLB is reliable, it is considered highly expensive and labor demanding thus not suitable for indexing large numbers of samples (Etxeberria 2007). To resolve this problem, a rapid, simple field diagnostic iodine test that could be used to prescreen samples intended for PCR analysis has been developed (Taba et al 2006). Anatomical studies conducted in the 1960s, showed "massive accumulation" of starch in leaf samples from HLB infected sweet orange trees (Etxeberria et al 2009). The accumulation of starch in HLB infected leaves has been found up to six times more than healthy leaves. Since the starch readily reacts with iodine, resulting in a very dark-grey to black stain, thus this technique has been adapted as one of the easiest diagnostic tool for HLB. These diagnosis methods have been reported to be more than 90% of agreement between PCR analysis and starch tests with iodine (Etxeberria 2007). Iodine solutions products labeled as either "tincture of iodine" or "iodine tincture" in 1 to 10 with water can be used.

Takushi et al (2007) also showed scratch method for HLB detection in citrus leaves using iodine-starch reaction. They found average quantity of starch was 514.2 mg/kg in HLB infected leaves and 85.6 mg/kg in healthy leaves (Welch's *t*-test $p<0.01\%$), a significant difference in quantity of starch between disease and healthy leaves. Based on this result, they devised Scratch method that uses sandpaper for HLB diagnosis. Accuracy of the scratch method diagnosis in field showed more than 90% of agreement rates to PCR assay. Chamberlain and Irey (2008) compared starch-based field test for HLB to the results from real-time PCR for testing of field samples from 1759 suspected symptomatic trees. They noted that 85% of the samples were positive by RT-PCR versus 78% positive for the starch test. Therefore, they recommended that the starch test should be considered a useful tool for HLB diagnosis in the field, but not as a substitute for PCR-based testing.

## Spectroscopy

In recent years, especially after detection of HLB in Brazil and Florida, USA several studies have been initiated to develop more efficient and cost-effective HLB detection techniques using spectroscopy. In a feasibility evaluation Sankaran et al (2011) found about 92% accuracy when they applied visible-near infrared spectroscopy technique for field detection of HLB in citrus trees using spectral reflectance data from the wavelength range of 350–2500 nm.

Hawkins et al (2010) found fourier transform infrared–attenuated total reflection (FT-IR-ATR) spectroscopy as a potentially suitable and inexpensive technique for rapid and early detection of HLB. The mid-infrared region of the spectrum reveals dramatic changes that take place in the infected leaves when compared to healthy non-infected leaves. The carbohydrates that give rise to peaks in the 900–1180 $cm^{-1}$ range are reliable in distinguishing leaves from infected plants versus non-infected plants. Furthermore, airborne

multispectral and hyperspectral imaging technology has also been tested recently for rapid detection of potentially diseased trees over a large area using hyperspectral imaging software (ENVI, ITT VIS) for data analysis with 87% accuracy (Kumar et al 2012).

Manjunath et al (2015) tested a unit Smart-DART™ connected to an Android device to detect Liberibacter in psyllids which is portable, affordable, sensitive and rapid to test. According to them with this device Psyllids can be tested to assess the prevalence of *Liberibacter asiaticus* in a population and for early detection of HLB in new areas being invaded by the psyllid.

In Barazil, Jarbas Caiado de Castro Neto, and his research team, spent one and a half years developing an inexpensive and portable way to detect citrus greening before the trees exhibit visible symptoms (Freshplaza, 2014). As mentioned in this report the end product is about the size of a credit card scanning machine, contains a laser, a spectrometer, and a processor to analyze the results. The principle behind this technique is that the diseased and healthy orange tree leaves emit different fluorescence spectra. Leaves affected by HLB emit more light in the middle of the visible spectrum, but have a lower peak - around 750 nm associated with the degradation of chlorophyll in the leaves. The spectrographic signature of citrus greening disease also appears almost immediately after a tree is infected, compared to the year or more it takes for visible symptoms to develop and this portable spectroscopic system called Agricultural Optronics Systems (AgriOS), is reported to detect citrus greening with greater than 95% accuracy (Freshplaza 2014).

## ECONOMIC LOSS

It is well known that HLB is one of the most serious citrus diseases worldwide, because once a tree is infected there is no cure and its yield is greatly reduced while the disease spreads rapidly. When, HLB becomes endemic, the disease progresses throughout the tree canopy and in the orchard very fast thereby reduce the economic life of affected orchards (Bove 2014). In many countries where HLB is present, it becomes a limiting factor for citrus production. It has been difficult to convince citrus farmers and regulatory agencies to take part in HLB management programs, because HLB management is based on elimination of diseased trees and reduction of vector population demands continuous efforts which is a costly undertaking (Bassanezi and Bassanezi 2008). Economic losses caused by HLB have been estimated in many countries and literatures on the importance and impact of the disease are available. Models that describe the disease incidence progress, disease severity progress and disease severity-fruit yield relationship according to the age of trees are also developed (Stuchi et al 2011, Roistacher 1996). In younger trees, the disease incidence and severity progress is faster than in older ones (Lopes et al 2011). Bassanezi et al (2011) have developed a mathematical model to describe HLB severity and yield relationship. According to this model HLB severity ($x$) of a grove allows prediction of the expected yield ($y$) using the formula: $y = \exp(-1.8x)$, $r = 0.33$.

Since the 1960s, Thailand has been plagued with HLB and has had a large impact on Thailand's citrus production. Under the presence of HLB, at the end of 8 years, a farmer would be losing $1,482 per acre, whereas if the grove was able to survive to year 10, a profit of approximately $1,370 per acre could be realized (Roistacher 1996). According to him in Thailand, profit is not realized unless trees survive to reach 10 years, as it takes time to recover the costs of planting and caring for the tree until production revenue is high enough to cover costs. The key problem was that in some regions of the country, groves were only lasting 6 to 8 years leading to 10 to 15% tangerine trees destruction each year. Indonesia was severely affected by HLB between 1960 and 1970 with an estimated loss of 3 million adult trees and much higher number of younger nursery plants (Gottwald et al 2007). By early 1960's the Philippines citrus plantation were estimated to cover 25,000 hectares. Ten years later 5 million trees were affected by HLB and the area planted to citrus was reduced to 60% (Aubert 1992).

The economic impact of HLB in Florida has also been assessed. HLB was first detected in Florida in 2005 and by 2014 this disease has caused significant economic and production losses costing additional US$ 750/acre for HLB management (FreshPlaza 2014). Hodges and Spreen (2012) estimated that a reduction of 23% in orange production during 2006 and 2010 was attributed to the presence of HLB. As a consequence, Florida's economy suffered a $3.9 billion loss resulting to total output, total value added and labor income decrease.

In Paraguay, HLB was first detected in March 2013. To prevent the spread of disease about 600,000 adult citrus plants and approximately 2,500,000 citrus saplings belonging to some 320 nursery owners were destroyed within two years in Paraguay costing about one million US$ to government as compensation to farmers for destroyed trees (FreshPlaza 2014a).

Quality of fruit juice is also affected by HLB. Goodrich-Schneider et al. (2008) analysed sweet orange juice hedonically for overall acceptability of flavor and sweetness among consumer panels (n=100). The juice from greening-affected fruit was always significantly less acceptable ($P<0.05$) than juice from healthy (control) fruits. Juice from greening-affected fruit was rated as significantly less sweet and significantly lower in orange flavor than juice from control fruit for all panels ($P<0.05$). Juice expressed from visually normal fruit from HLB affected trees generally fell between control juice and greening-affected fruit juice in terms of overall acceptability, sweetness and orange flavor.

## HOST RANGE

Huanglongbing is a disease of rutaceous plants (Bove 2014). None of the citrus fruit species are resistant to HLB (Bove 2006). However, responses of citrus species to '*Ca.* L. asiaticus' has been found variable. It severely affects sweet orange, mandarin and tangelo trees but many other species show more or less pronounced symptoms of the disease. Mexican lime (*Citrus aurantifolia*) is less susceptible than sweet orange and mandarin even though it is a preferred host of the vector Diaphorina citri (Roastcher 1996). Based on severity of HLB symptoms and the ability to continue growth of the plants inoculation with '*Ca.* L. asiaticus' Folimonova et al (2009) grouped citrus genotypes into four categories as (i) sensitive: *C. halimii*, Nules clementine mandarin, Minneola tangelo, sweet oranges and grapefruit (ii) moderately tolerant: Sun Chu Sha mandarin, sour orange, volkamer lemon, *C. macrophylla*, wingle citrumelo, citron, Palestine sweet lime, acid lime, calamondin, and *C. micrantha* (iii) tolerant: Eureka lemon, Persian lime, Carrizo citrange, and *Severinia buxifolia* (iv) variable (some branch sensitive and some branch tolerant): pummelos, *C. amblycarpa*, cleopatra mandarin, *C. indica*, and

meiwa kumquat. Ahmad et al. (2011) also classified citrus species into very susceptible, susceptible and tolerant categories based on reaction with HLB pathogen.

## MANAGEMENT STRATEGIES

### Inoculum Reduction and Vector Control

Currently, there is no established cure for this century-old emerging disease. There is general consensus throughout the world on three general management practices for the management of the disease; the planting of certified clean planting materials, effective control of its vector psyllid populations and removal of infected trees that serve as an inoculums source for psyllid acquisition (Bove 2006). These management practices must be adopted in order to have a successful citrus greening management program (Stuchi et al 2011). However, HLB management is difficult, if inoculum sources are widespread and the psyllid vector is well established.

There are no HLB tolerant commercial citrus species and cultivars at present to replace declining healthy trees (Bove 2006). The general control strategy has been to eradicate all existing sources of HLB within an area, then replant with HLB-free trees grown from clean budwood. Psyllid populations must also be reduced as much as possible. Biological control of the psyllid vector is only possible in locations that do not favour build-up of psyllid populations and is often compromised when hyper-parasites are present.

Preventing HLB from entering to healthy area is much easier than trying to eradicate or control it. It is important to avoid bringing propagation materials from HLB-infected areas to non infected area (Abdullah 2009). Zhang et al. (2011) has described various situations on which removal of infected trees and replanting is feasible. One strategy is to immediately reset trees that were removed. However, since psyllids are attracted to new growth and young trees flush more frequently than immature trees, young trees in a block with mature trees supporting psyllids are at greater risk than solid set young trees in a newly-planted block. Pruning may also be an extremely hazardous practice in areas under high HLB pressure, if a careful vector control program is not applied (Stuchi et al 2011) because pruning enhance new flush growth which harbor psyllids. Another strategy is to continue to remove infected trees until reduced tree numbers make the block economically unproductive, then the remove remaining trees and the entire block is replanted (Zhang et al., 2011).

Stuchi and Girardi (2011) have given detail information on various factors associated with the success or failure of HLB management based on inoculum reduction and control of psyllid vector populations by insecticide treatments adopted in Brazil. According to them HLB management practices adopted in Brazil are (i) selection of naturally occurring tolerant materials (ii) new regions for citrus production (iii) screen-house nursery trees production (iv) use of repellent and attractive plants for vector (v) protected cultivation (vi) intercropping and ultra high density (UHD) plantings (vii) intensive production systems (viii) the use of larger nursery trees.

### Chemical Control

Zhang et al (2011) reported that the combination of penicillin and streptomycin (PS) was effective in eliminating or suppressing the Las bacterium and provided a therapeutically effective level of control for a much longer period of time than when administering either antibiotic separately using a propagation test system with Las-infected periwinkle and citrus plants. However, Bove (2006) suggested that antibiotic treatment has only short term effect and therefore, is not a sustainable approach in HLB control.

### Nutrition

The use of nutritional applications to control or offset the deleterious effects of HLB has been a topic of considerable discussion and debate (Spann et al 2011). However, most reports are anecdotal and without sufficient statistical validity. To develop empirical evidences Gottwald et al (2011) compared combinations of nutritional components consisted of phosphite with Mn-carbonate, Mn-metalosate, Cu-metalosate, or Zn-metalosate, and injection treatments using soluble copper or silver mixed with a polymer along with a control consisting of a standard insecticide program for psyllids. There was no significant difference in titer dynamics, yield (number fruit per tree, kg fruit/tree, proportion of fruit dropped), or quality (Brix, acid, brix:acid ratio), compared to the control. The additional nutrition did not sustain tree health, yield, or fruit quality. Rather, there is a major concern that nutritional supplements may promote area-wide inoculums buildup and spread (Gottwald et al 2011).

Practice of adding additional nutrition, especially the micronutrients expecting extended productive life of infected trees has been a common practice in China (Spann et al 2011). But review of 60+ years' history of research and field practice in China reveals no consistent evidence to support the notion that nutrient management can maintain productivity of HLB-infested trees over the long term (Xia and Sequeira, 2011). Relationship of plant nutrition and HLB infection has been studied in four commercial citrus groves in Florida (Spann et al 2011). Preliminary results of the research showed that HLB-infected trees are consistently deficient in Ca, Mg, Mn, Zn and B, and in an orchard, these nutrient deficient trees are more likely to be HLB-infected than the nutrient sufficient trees. The researchers are not yet clear whether these deficiencies were a result of decreased nutrient uptake, or if the nutrients were being bound within the plant making them unavailable. HLB-affected trees characteristically suffer from a damaged root system and nutritional disorder because of interrupted transport of photo-assimilates from shoot to the root, and mineral nutrients/water from root to the shoot (Spann et al 2011).

He et al (2011) conducted greenhouse experiments to examine the effects of growth-priming agents including root growth enhancer, trace element combination (Zn, Cu, and Mn), plant growth regulators, and sugar transporter alone or in combination on HLB-affected citrus seedlings. When applied alone, root growth enhancer was most effective in promoting plant growth followed by trace element combination as indicated by a significant increase in shoot and root biomass, particularly root length and surface area, as compared with the control (He et al 2011). The main cause of visible HLB symptoms, yield reduction, and tree decline appears to be disruption of phloem tissue, which blocks the flow of photosynthate and nutrients from source to sink tissue (Bove 2006). If supplemental nutrition is

a sustainable approach, it is expected that foliar nutrients will reduce or eliminate damage and plugging in citrus phloem tissue caused by the bacterium and possibly reduce spread or replication of the bacterium in infected trees (He et al 2011).

### Use of Tolerant Rootstocks

In Barazil, 12 rootstocks namely two Rangpur limes (FCAV and Limeira), three trifoliate oranges [Rubidoux, FCAV, and Flying Dragon (FD)], *Swingle citrumelo*, Sunki, and Sun Chu Sha Kat mandarins, *Orlando tangelo*, *Carrizo citrange*, and the hybrids Changsha × English Small and Rangpur lime × *Swingle citrumelo* have been tested for HLB reaction (Stuchi et al, 2011). After a first 7-year evaluation cycle for HLB infection the average cumulative incidence (CI) of HLB was 72.1%, with CI values varying from 10 to 100%. For most of the rootstocks, CI values were equal or higher than 60%, while the trees budded onto the FD rootstock showed the lower HLB incidence (10%). Albrecht and Bowman (2012) used greenhouse inoculation tests and field trials to investigate the tolerance of some *Citrus* × *Poncirus* hybrids to infection of *Candidatus* Liberibacter asiaticus (Las). The citrus rootstock US-897 (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Raf.) was observed to be tolerant to HLB in field plantings (Albrecht and Bowman 2012).

### Guava Intercropping

Since long, famers from Mekong Delta of South Vietnam have been practicing interplanting of citrus with guava and those citrus orchards planted with guava showed much lower psyllid infestation levels and low incidence of HLB compared to citrus orchards lacking guava (Hall et al 2007). This practice adopted by Vietnamese farmers drew the attention of scientific communities of many countries during 1990s and various scientific studies were initiated to understand the scientific reasons. The first of this kind of research was a collaborative research projects involving Vietnamese, Japanese and Australian scientists (Beattie et al 2006). The findings of this research which was presented in a meeting held during December 2006 in Japanese International Research Center showed that interplanting citrus with guava negated infestations of Asian citrus psyllid and consequently HLB (Beattie et al 2006). This study collected the scientific information that young citrus interplanted with guava remained disease-free for a year whereas sole citrus crop showed signs of the disease within four months of planting and reached over 30% trees infected within a year (Hall et al 2007).

A team of American scientists who also made a observation tour to Vietnam in April 2000 noted that the normal life of sole citrus plantings in Mekon region of Vietnam was 2 to 4 years, but those interplanted with white guava were surviving for up to 15 years (Gottwald et al 2010). Although raising guava as an intercrop reduced psyllid population in citrus orchards (Hall et al 2007), the mechanism by which this occurs was unknown. It was postulated that the effects of guava on citrus psylla could be due to mechanical/physical disruption on host recognition, repellent effect of volatile compounds from guava or chemical alteration of the volatile compounds emitted by citrus reacting with guava compounds. From various studies conducted in Florida, USA to determine, if guava has a repellent effect on adults of Asiatic citrus psyllids. Gottwald et al. (2010) noted high adult mortality rates occurred when psyllids were confined to guava in no-choice situations, with 95% mortality occurring within 6-9 days. They postulated that the effect may be due to volatile compounds produced by guava that are deleterious to psyllids. In another study, Zaka et al (2010) evaluated the repellent effect of guava leaf and factors attributed to this activity, response of adult psyllids to guava leaves and its odor in cage test and Y-tube alfactometer test. The alfactometer response of adult psyllids to guava leaf odor was dose-dependent and both male and female psyllids responded similarly to the guava leaf odor.

Since more than 75% of the Florida citrus crops has been infected with HLB, USDA has provided 43 million US $ for its research (FreshPlaza 2015b). Funded projects include a grant to the University of Florida to develop a bactericide that can be applied to infected citrus trees to reduce or eliminate pathogens, a project at Kansas State University to develop a therapeutic delivery system that will prevent *Candidatus Liberibacter asiaticus* from infecting plants or prevent the development of HLB in infected citrus, and a grant at the University of California-Davis that focuses on using new genetic approaches to managing the Asian citrus psyllid that causes HLB (FreshPlaza 2015b).

## CONCLUSION

Review of scientific literatures revealed that HLB is the most devastating disease of citrus fruit crops. Most commercial citrus species of Nepal like mandarin and sweet orange are very susceptible to the disease while acid lime is slightly tolerant, but it is carrier of HLB bacterium serving as hidden source of innoculum. The disease is present in Nepal since early 1960s and has spread many commercial citrus pockets of the country over the years (Bove, 2006 & 2014, Regmi et al, 1996 & 2010, Regmi and Yadav, 2007). Past research efforts were found mainly focused on survey and disease indexing leaving inadequate attention to its long term management. It has resulted to massive decline of citrus orchards especially located below 1000 m altitude where population of vector – psyllid is abundant. Therefore, successful HLB management strategies particularly those of Brazil and Florida can be adopted in Nepal to save country's citriculture.

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